Industrial Engineering Journal
ISSN: 0970-2555
Volume : 53, Issue 8, No.1, August : 2024

INVESTIGATING THE ROLE OF M. TUBERCULOSIS ANTIGENS IN REGULATING THE IFN-Γ RECEPTOR LEVELS IN MOUSE MACROPHAGES

Anubha Jain Research Scholar, Department of Biomedical Sciences, New Delhi
Dr. Sonlata Bargotya Assistant Professor, Department of Chemistry, Government College, Tonk (Raj.)

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- γ R, in order to initiate proper signaling. IFN- γ R consists of two hetero dimeric subunits, IFN- γ R1 (ligand binding) and IFN- γ R2 (signaling subunit). The IFN- γ R 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- γ R1, in vitro experiments were designed, wherein the role of TLR pathway towards antigen mediated IFNγR1 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,

the effects appear to be strongest when multiple TLRs are impaired. Further experiments were done to identify intracellular secondary messengers mediating IFN-γ expression by the antigens using different inhibitors of various intracellular pathways. Experiments have shown that MyD88 regulates a protective immune response to M. tuberculosis in mice. Two key pathways that are targeted by M. tuberculosis in DCs (and also macrophages) are the calcium pathway that affects the survival and proinflammatory response generation from DCs and the cysteine protease pathway that largely effect antigen processing and presentation to T cells, thereby modulating priming of T cells early on in the infection process. Using calcium and cysteine protease pathway-specific siRNA libraries, we identified genes that play critical roles in modulating diverse functions of dendritic cells (DCs) during M. tuberculosis infection. Knockdown of many of these genes in the two pathways resulted in reduced bacterial burden within DCs. Therefore, in the light of the above, in this study, we elucidated the role of genes of these two pathways in regulation of IFN-γ expression with respect to M. tuberculosis infection by employing pathway-specific RNA libraries.

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 preantibiotic 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. Reinfection 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 hypotheses about host immunity.

AIM: To investigate the ability of M. tuberculosis antigens in regulation of intracellular IFN-γ receptors on macrophages

MATERIAL AND METHODS

Cell line used The J774 mouse macrophage cell line, cultured in RPMI supplemented with 10% heatin activated FBS, 1% sodium pyruvate, 0.1% beta mercaptoethanol at 37°C in 5% CO2.

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 day1 (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-γR, using fluorescein isothiocyanate-tagged antibodies, followed by incubation with streptavidin phycoerythrin, and analyzed by flow cytometry on FACSCalibur (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).

RESULTS AND DISCUSSION

To investigate the ability of M. tuberculosis antigens in regulation of intracellular IFN-γ receptors on macrophages

Industrial Engineering Journal
ISSN: 0970-2555
Volume : 53, Issue 8, No.1, August : 2024

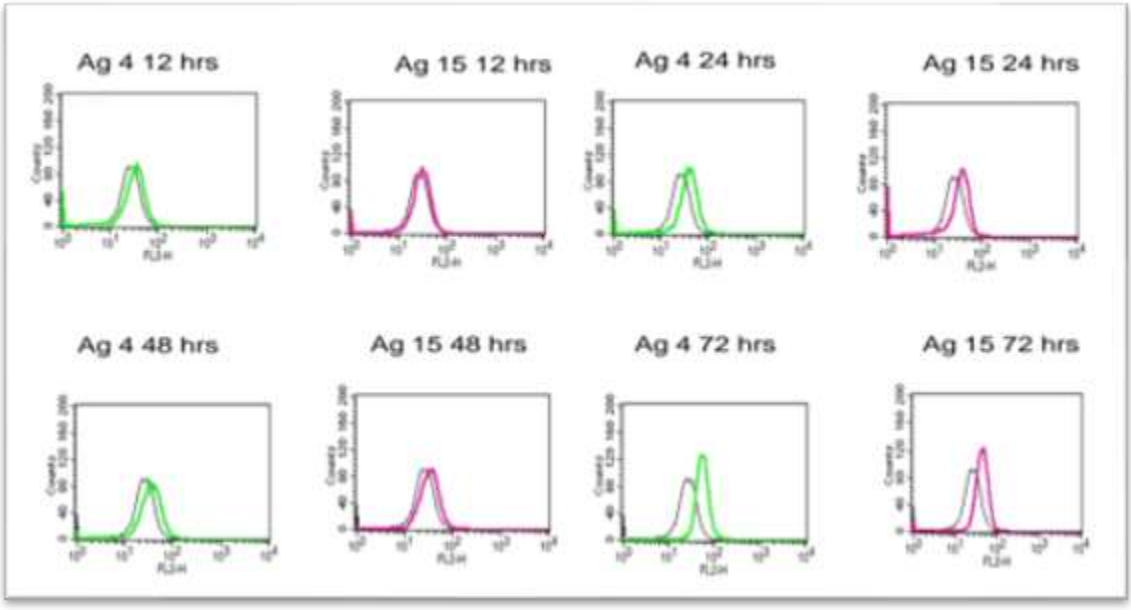

Fig1: Kinetics of IFNγ- receptor expression on the surface of j774 by RV2463 & RV3416

To determine whether addition of Rv2463 and Rv3416 changes cell-surface expression of the IFNγ receptor, mouse macrophage cell line J774 were in vitro stimulated with Rv2463 and Rv3416 and receptor expression was monitored by flow cytometry. IFN-γR1 was up-regulated on the surface of j774 with in 24 h with the lowest levels detected at around 12 h (Fig. 1). The upregulation of IFN-γR1 on these cells was time dependent and difference is much more pronounced at 72hrs. Since we could very well detect the up regulation even at 24hrs we used this time point for further studies.

Pathogens ability to modulate expression of IFN-R is not unique. Increases and decreases in tokine receptor expression have been demonstrated with a variety of pathogens. Mycobacterium and Trypanosoma cruzi have been reported to down-regulate IFNR expression on murine macrophages and B cells, respectively, while Legionella has been shown to increase expression of both II-IR and TNF receptor on macrophages Changes in cytokine responsiveness by modulation of the expression of cytokine-specific receptors are one means by which a pathogen could manipulate cell-mediated immune responses over of Rv2463 and Rv3416 up-regulates the expression of IFN-y receptors upon M. tuberculosis infection. Furthermore, these antigens increase the IFN-y receptor expression significantly higher when stimulated alone in J774 macrophage cell line. The increases in cytokine receptor expression induced by these antigens are facilitated by the TLR system. Decrease in IFN-R expression upon knockdown of MyD88, IRAKI, IRAKM, TRAF6 by Rv3416 signifies the importance of TLR signalling. Rv2463 did not show any change in expression of IFN-R with MyD88, IRAKI and IRAKM knockdown. But TRAF6 silencing could be seen as blocking up- regulation of IFN-YyR expression by Rv2463, which indicates that MyD88 independent pathway might be involved. TRAF6 seems to be the nodal point where the signal converges from Rv2463 and Rv3416. TRAF6 signals multiple downstream pathways. One of them is activation of atypical PKCs. Our result indicates that novel and conventional PKCs which were activated by extracellular calcium plays negative role in IFN-yR upregulation. So probably TRAF6 signals these atypical PKCs which were calcium and DAG independent and then these atypical PKCs might activate NF-kB which was reported to be essential for IL-If-mediated up-regulation of IFN-YR. It's important at this point to look at IFN-y signalling IFN-y exerts its effect on cells by interaction with IFN-YR. Binding of IFN-y to IFN-YR activates JAKI and JAK2 which phosphorylates STATI at Tyrosine 701 which can now go into nucleus and bind promoter. However, for full transcription activation it requires to be phosphorylated at Serine 727 also, which were mediated by PKC-delta and Calcium/Calmodulin-dependent protein kinase. So,

though M. tuberculosis increases the IFN-YR by Rv3416 and Rv2463, it puts the check over IFN-7 mediated signalling.

It is tempting to speculate the role IFN-y Receptors plays during Rv3416 and Rv2463 expression by M. tuberculosis. One possibility is that these receptors could function as decoys. IFN-y would hind to these receptors but not activate signal transduction, thereby reducing the local concentration of IFN-y. Secondly, these receptors might bind to intracellular IFN-y which was also seen to be increasing with time, there by providing an anti-apoptotic signal resulting in prolonged survival of M. tuberculosis in the face of an otherwise effective immune response.

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 SH. 2000. Exploiting the immune system goward new vaccines against intracellular bacteria. Ade Immunol, 75-88.
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 uberculosis Infection. Immunology and Cell Biology, 1-6.
3. Singhal A. Jaiswal A, Arora V, and K. Prasad H. May 2007. Modulation of Gamma Interferon Receptor 1 by Mycobacterium tuberculosis: a Potential Immune Response Evasive Mechanism. Infection and immunity, 2500-2510.
4. Gupta D. Sharma S, Singhal J. T. Satsangi A, Antony C. and Natarajan K. Apr 12, 2010. Suppression of TLR2-induced IL-12. Reactive Oxygen Species, and Inducible Nitric Oxide Synthase Expression by Mycobacterium tuberculosis Antigens Expressed inside Macrophages Baring 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. Singhal J, Agrawal N, Vashishta M. Gayatri Priya N. K. Tiwari B. Singh Y, Raman R. and Natarajan K. March 30, 2012 Suppression of Dendritic Cell-mediated Responses by Genes in Calcium and Cysteine Protease Pathways during Mycobacterium tuberculosis Infection. The journal of biochemistry,11108-11121.
7. Raviglione, M. C. Snider D. E. Jr. and Kochi A. 1995 Global epidemiology of tuberculosis: Barbidity and mortality of a worldwide epidemic. JAMA. 273 220-226.
8. Turett. G. S. Telzak E. E, Torian L. V. S. Blum, Alland D. Weisfuse I, and Fazal B. A. 1995. Improved outcomes for patients with multidrug-resistant 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. J.A.M.A., 698-702.
10. Stead. W. W. 1995. Management of health care workers after inadvertent expesure to uberculosis: a guide for the use of preventive therapy. Ann Intern. Med, 122906-912.
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 lated glycoconjugates: structure, biogenesis and role in Mycobacterium tuberculosis mysiology and host-pathogen interaction. FEMS Microbiology Reviews, 1126-1157.
13. Kleinnijenhuis, Oosting M, Joosten L. A. Netea M.G. 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. Tania Botha, Drennan M. Soler M.N. Le Bert M. Schnyder B, and Ryffe B. 2004, Toll-like septor pathways in the immune responses to mycobacteria. Microbes and Infection, 946-959.