INVESTIGATION OF THE ROLE OF ESX5 ASSOCIATED *MYCOBACTERIUM TUBERCULOSIS* PE18 AND PPE26 PROTEINS DURING THE HOST-PATHOGEN INTERPLAY

BY

AQUIB EHTRAM

KUSUMA SCHOOL OF BIOLOGICAL SCIENCES

INDIAN INSTITUTE OF TECHNOLOGY DELHI

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AQUIB EHTRAM

Kusuma School of Biological Sciences

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CERTIFICATE

This is to certify that the thesis entitled **"Investigation of the role of Esx5 associated *Mycobacterium tuberculosis* PE18 and PPE26 proteins during the host-pathogen interplay"**, submitted by **Mr. Aquib Ehtram** to the **Indian Institute of Technology Delhi**, New Delhi, for the award of the degree of **Doctor of Philosophy** in Kusuma School of Biological Sciences, is a record of bonafide research work carried out by him.

Mr. Aquib Ehtram has worked under my guidance and supervision and has fulfilled the requirements for the submission of the thesis. The research report and results contained in the thesis have not been submitted in part or in full to any other university or institute for the award of any degree or diploma.

New Delhi
September 2022

Prof. Seyed E. Hasnain
Kusuma School of Biological Sciences
Indian Institute of Technology Delhi
Hauz Khas, New Delhi-110016

Prof. Vivekanandan Perumal
Kusuma School of Biological Sciences
Indian Institute of Technology Delhi
Hauz Khas, New Delhi-110016

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Abstract

ESX-5 genomic locus of *Mycobacterium tuberculosis (M. tb)* comprises five *pe/ppe* genes. Key attributes of these proteins are the formation of secretion competent heterodimer recognized by a cognate chaperone. PE/PPE proteins have been reported to play a role in a range of diverse functions including immune modulation, cell death regulation, cooption of porin function, and host-pathogen interaction. *M. tb* exploits this enigmatic family of proteins to overcome host immunity. In the present work, the physical interactions between PE18 with PPE26, PE18 with PPE27, and PE19 with PPE25 has been demonstrated. It was also shown that the cognate chaperone EspG5 recognized PPE proteins PPE25, PPE26, and PPE27 showing possible functional cooperation. Interestingly, it was further shown that PE18 and PPE26 are co-operonic in *M. tb*. Most PE and PPE proteins localize to the outer cell envelope and are employed by *M. tb* to manipulate host-immune functions. Intriguingly, recombinant *Mycobacterium smegmatis* expressing PE18, PPE26, and PE18+PPE26 displayed enhanced intracellular survival inside macrophages. Using macrophage cells and mice as an experimental model, it was deciphered that PE18 and PPE26, proteins and recombinant *M. smegmatis* elicited production of pro-inflammatory cytokines (PCs) IL12, TNFα, IL6, IL-1β, and IFNγ. It was also observed that secretion of PCs is mediated by TLR2 and adapter Myd88. These proteins elicited expression of costimulatory molecule CD80 and activation markers MHC-I and MHC-II on the surface of peritoneal macrophages, suggesting M1 polarization. Recombinant PE18 and PPE26 proteins generated robust effector memory response mediated by CD4+ and CD8+ T-cells. This is evidenced by the increased level of CD44 and reduced expression of CD62L. PE/PPE proteins are well-known inhibitors of phagosome maturation. To unravel the role of PE18 and PPE26 in actin dynamics, endosome, and phagosome maturation, recombinant *M. smegmatis* containing *pe18*, *pe26*, and *pe18+ppe26* genes

and vector alone were used to infect macrophages. Results presented in the present thesis strongly suggested that recombinant *M. smegmatis* antagonistically regulated early and late endosome markers RAB5 and RAB7. The downregulation of early endosome marker EEA1, indicated a hampered endosome-phagosome maturation dynamic. Experimental evidence presented in this thesis showed that recombinant *M. smegmatis* expressing PE/PPE proteins PE18 and PPE26 downregulated the production of actin nucleation and polymerization effectors RAC1, N-WASP, ARP2, and Profilin, suggesting impaired actin dynamic. Inhibition of actin dynamics points toward the roles of these proteins in phagosome maturation arrest. These findings implicate that PE/PPE proteins PE18 and PPE26 are exploited by *M. tb* for manipulating host-pathogen interaction to subdue host immunity for efficient survival and pathogenesis.

सार

माइकोबैक्टीरियम ट्यूबरकुलोसिस (एम. टी बी) के ESX-5 जीनोमिक स्थान में पांच PE/PPE जीन शामिल हैं। इन प्रोटीनों की प्रमुख विशेषताएं एक कॉग्नेट चैपरोन द्वारा मान्यता प्राप्त स्रावी सक्षम हेटेरोडिमर का निर्माण हैं। PE/PPE प्रोटीन को विभिन्न कार्यों की एक श्रृंखला में भूमिका निभाने के लिए सूचित किया गया है, जिसमें प्रतिरक्षा मॉडुलन, कोशिका मृत्यु विनियमन, पोरिन फ़ंक्शन का कोप्शन, और मेजबान-रोगज़नक़ बातचीत शामिल है। *M. tb* मेजबान प्रतिरक्षा को दूर करने के लिए प्रोटीन के इस गूढ़ परिवार का शोषण करता है। वर्तमान कार्य में, PE18 के साथ PPE26, PE18 के साथ PPE27 और PE19 के साथ PPE25 के बीच भौतिक अंतःक्रियाओं का प्रदर्शन किया गया है। यह भी दिखाया गया कि कॉग्नेट चैपरोन EspG5 ने संभावित कार्यात्मक सहयोग दिखाते हुए PPE प्रोटीन PPE25, PPE26 और PPE27 को मान्यता दी है। दिलचस्प बात यह है कि आगे यह दिखाया गया कि PE18 और PPE26 *M. tb* में सहकारी हैं। अधिकांश PE और PPE प्रोटीन बाहरी सेल लिफाफे में स्थानीयकृत होते हैं और *M. tb* द्वारा मेजबान-प्रतिरक्षा कार्यों में हेरफेर करने के लिए नियोजित होते हैं। दिलचस्प बात यह है कि PE18, PPE26, और PE18 + PPE26 को व्यक्त करने वाले पुनः संयोजक *माइकोबैक्टीरियम स्मेग्मेटिस* ने मैक्रोफेज के अंदर बढ़े हुए इंट्रासेल्युलर अस्तित्व को प्रदर्शित किया। एक प्रायोगिक मॉडल के रूप में मैक्रोफेज कोशिकाओं और चूहों का उपयोग करते हुए, यह समझा गया कि PE18 और PPE26, प्रोटीन और पुनः संयोजक *M. smegmatis* ने प्रो-इंफ्लेमेटरी साइटोकिन्स (PC) IL12, TNFα, IL6, IL-1β और IFNγ का उत्पादन किया। यह भी देखा गया कि PC के स्राव की मध्यस्थता TLR2 और एडेप्टर Myd88 द्वारा की जाती है। इन प्रोटीनों ने पेरिटोनियल मैक्रोफेज की सतह पर कॉस्टिम्युलेटरी अणु CD80 और सक्रियण मार्कर MHC-I और MHC-II की अभिव्यक्ति प्राप्त की, जो M1 ध्रुवीकरण का सुझाव देते हैं। पुनः संयोजक PE18 और PPE26 प्रोटीन ने CD4+

और CD8+ T-कोशिकाओं द्वारा मध्यस्थता से मजबूत प्रभावकारी स्मृति प्रतिक्रिया उत्पन्न की। इसका प्रमाण CD44 के बढ़े हुए स्तर और CD62L की घटी हुई अभिव्यक्ति से है। PE/PPE प्रोटीन फागोसोम परिपक्वता के प्रसिद्ध अवरोधक हैं। एक्टिन डायनेमिक्स, एंडोसोम, और फागोसोम परिपक्वता में PE18 और PPE26 की भूमिका को जानने के लिए, पुनः संयोजक *M. smegmatis* जिसमें *pe18, pe26,* और *pe18+ppe26* जीन और अकेले वेक्टर हो, का उपयोग मैक्रोफेज को संक्रमित करने के लिए किया गया था। वर्तमान थीसिस में प्रस्तुत परिणामों ने दृढ़ता से सुझाव दिया कि पुनः संयोजक *M. smegmatis* विरोधी रूप से प्रारंभिक और देर से एंडोसोम मार्कर RAB5 और RAB7 को नियंत्रित करता है। प्रारंभिक एंडोसोम मार्कर EEA1 के डाउनरेगुलेशन ने एक बाधित एंडोसोम-फागोसोम परिपक्वता गतिशील का संकेत दिया। इस थीसिस में प्रस्तुत प्रायोगिक साक्ष्य से पता चला है कि PE/PPE प्रोटीन PE18 और PPE26 को व्यक्त करने वाले पुनः संयोजक *M. smegmatis* ने एक्टिन न्यूक्लिएशन और पोलीमराइजेशन इफेक्टर्स RAC1, N-WASP, ARP2 और प्रोफिलिन के उत्पादन को कम कर दिया, जो बिगड़ा एक्टिन डायनेमिक का सुझाव देता है। एक्टिन गतिकी का निषेध फागोसोम परिपक्वता गिरफ्तारी में इन प्रोटीनों की भूमिकाओं की ओर इशारा करता है। इन निष्कर्षों से पता चलता है कि कुशल अस्तित्व और रोगजनन के लिए मेजबान प्रतिरक्षा को वश में करने के लिए PE/PPE प्रोटीन PE18 और PPE26 का *M. tb* द्वारा शोषण किया जाता है।

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List of acronyms

TB	**Tuberculosis**
HIV	**Human immunodeficiency virus**
BCG	**Bacillus Calmette Guerin**
M. tb	***Mycobacterium tuberculosis***
M. smeg	***Mycobacterium smegmatis***
PE	**Proline-Glutamate**
PPE	**Proline-Proline-Glutamate**
PGRS	**Polymorphic GC rich repeat sequences**
MPTR	**Major polymorphic tandem repeats**
ESAT6	**6 kDa Early secretory antigenic target**
MDR	**Multi drug resistant**
XDR	**Extensive drug resistant**
MOI	**Multiplicity of Infection**
PAMPs	**Pathogen associated molecular patterns**
PRRs	**Pathogen recognition receptors**
TLRs	**Toll like receptors**
NO	**Nitric oxide**
iNOS	**Inducible nitric oxide synthase**
OD	**Optical Density**
CFUs	**Colony forming units**
TNF	**Tumor necrosis factor**

IL-12	**Interleukin-12**
IL-6	**Interleukin-6**
IL-1β	**Interleukin-1 beta**
IFN- γ	**Interferon gamma**
WHO	**World Health Organization**
WT	**Wild type**