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**Paper Poster Session**
**New treatment options for mycobacterial infections**

# Antibodies targeting PE_PGRS33 neutralize the ability of Mycobacterium tuberculosis to entry macrophages

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**Background:** *Mycobacterium tuberculosis* (*Mtb*) PE_PGRS33 is a surface-exposed protein that has been implicated in tuberculosis pathogenesis. PE_PGRS33 was shown to interact with TLR2 on host macrophages and to induce inflammatory signals that promote cell death. More recently, an *Mtb* strain where the pe_pgrs33 gene was inactivated, was shown to be impaired in its ability to entry in murine and human macrophages and also in this case the PE_PGRS33-mediated entry of *Mtb* was dependent upon interaction with TLR2. In this context, PE_PGRS33 could emerge as a potential target of a humoral response specifically aimed at hampering key processes in TB pathogenesis. The aim of this study is to assess the impact that antibodies, specifically raised with the native form of PE_PGRS33, can have on *Mtb* infection.

**Material/methods:** A system to express and purify PE_PGRS33 protein under native condition was developed and the protein biological activity was measured in *in vitro* assays, by measuring the secretion of cytokines by murine macrophages or by assessing the ability to complement the *Mtb*Δpe_pgrs33 phenotype. Purified PE_PGRS33 was also used to immunize Balb/c mice and the anti-PE_PGRS33 serum specificity was assessed in immunoblots using whole MTBC complex cell lysates. The neutralizing ability of the anti-PE_PGRS33 serum was tested in *in vitro* assays where murine macrophages were infected with *Mtb* and *Mtb*Δpe_pgrs33 strains and intracellular bacilli determined by CFU counting.

**Results:** PE_PGRS33 protein was successfully expressed and purified in mg amount under native condition in *E. coli*. The recombinant PE_PGRS33 protein was shown to elicit TNF-α when added to murine and human macrophages, indicating that it retained the native functional and biological properties. The antiserum obtained from mice immunized with the purified protein was able to detect only a band corresponding to PE_PGRS33 on MTBC cell lysates, demonstrating a high specificity and indicating that the serum raised with the native protein does not react with other PE_PGRS proteins. We also demonstrated that the anti-PE_PGRS33 polyclonal serum was able to reduce *Mtb* entry into macrophages and that this reduction was not observed in the *Mtb*Δpe_pgrs33 strain. Moreover, we showed that adding the protein to the *Mtb*Δpe_pgrs33 strain during macrophages infection *in vitro* restores the wild type phenotype of *Mtb* entrance in macrophage and the antiserum can neutralize the activity of PE_PGRS33.

**Conclusions:** The results of this study indicate that antibodies raised with the native form of PE_PGRS33 can neutralize the biological activity of this protein, which plays a key role in *Mtb* entry in macrophages and in immunomodulation during infection. As such, PE_PGRS33 could be a potential target of future vaccination strategies against TB.