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New insights into the host cell necrosis in tuberculosis

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Despite the years of intensive research tuberculosis still remains a major global health hazard. In 2011 there were ca. nine million new cases of tuberculosis and ca. 1.5 million associated deaths, most among the poorest persons in both high-income and developing countries.[1-2] Both treating tuberculosis and preventing new infections has proven to be cumbersome. The Bacillus Calmette-Guérin (BCG) vaccine was developed nearly a century ago in France,[3] but it still remains the only vaccine available against TB in clinical use. While this attenuated, live *Mycobacterium bovis* based vaccine protects children against disseminated TB it does not effectively protect against adult pulmonary TB, or prevent the reactivation of latent TB infection. Notably, BCG has also been associated with severe complications in newborns, including lymphadenitis, abscess and osteitis.[4] Both active and latent TB are currently treated with a combination of antibiotics, such as insoniazid, rifampisin, ethambutol and pyrazinamide.[2] Unfortunately with the raise in the incidence of multidrug-resistant (MDR) infections the first-line treatments often fail to cure the disease. Treating MDR requires substantially longer treatment periods that are associated with a high risk of intolerance and toxic side effects. Taking aforementioned into account, it is evident that we will need better preventive means and therapeutics to battle TB. In order to accomplish these goals we will need more information about the molecular mechanisms that underlie beyond the immunogenicity and pathogenic behavior of the *Mycobacterium tuberculosis* bacterium.

One viable strategy to find the Achilles' heel of *Mycobacterium tuberculosis* is to explore its virulence factors. Mtb virulence is controlled by a plethora of bacterial genes that contribute to mycobacterium's metabolism, structure, secretion systems or regulate the host responses especially in alveolar macrophages and lung epithelial cells.[5] For example, previous results have suggested that first Mtb inhibits apoptosis in infected host cells, which is beneficial for its intra-cellular growth. However, at a later stage of infection virulent mycobacteria can also promote host cell necrosis, which is considered a cell exit strategy for Mtb.

In this issue of *Virulence* Danelisvili et al.[6] use an unbiased screening approach to explore genes that regulate the host cell necrosis in *Mycobacterium tuberculosis* infected macrophages. Authors take advantage of a large collection of Mtb transposon mutants (5000 clones) and initially identify 7 bacterial clones that induce significantly less cell death in a human THP-1 macrophage cell line. Bacterial clones that show unaltered intra-cellular growth are kept for further studies and initially assessed for their ability to induce necrotic versus apoptotic cell death. Previous studies have demonstrated that the Mtb-induced cell necrosis and consequent exit from the infected cells is associated with the activation of the intrinsic apoptotic pathway through mitochondrial damage. Five identified necrosis deficient *Mycobacterium tuberculosis* mutant clones (NDM) showed a delayed capacity to activate the intrinsic apoptotic pathway, which provides a mechanistic explanation for the observed reduction in necrotic cell death.

Authors go on to identify the mutated genes in the NDM clones by non-specific nested suppression PCR method. These efforts remarkably show that 2 out of 5 NMDs bear an interruption in the same gene, Rv3873, which then becomes the focus for further studies. Rv3873 encodes for PPE68, which is a part of the cell envelope in the H37Rv Mtb strain with reported immunogenicity properties and functions in the secretory processes.[7] Interestingly, previous studies have also demonstrated that PPE68 is located in the RD1 region found in both *M. marinum* and *M. tuberculosis*. The RD1 region controls the pathogenesis of tuberculosis via e.g. the ESAT-6 and CFP-10 secretion, both of which are associated with a number of virulence related mechanisms, including host cell necrosis.

To gain further insight into the molecular mechanisms authors perform comprehensive profiling of the PPE68 interactome by using pull-down assays and mass spectrometry. These studies led to the identification of several novel PPE68 interacting proteins, some of which some were kept for confirmatory assays using the yeast-2 hybrid system. The confirmed PPE68-interactors were finally analyzed for their ability to induce macrophage necrosis in elegant experiments using protein over-expression and genetic deletion together with rescued protein expression. These data unequivocally showed that a novel PPE68-interactor, hypothetical protein Rv2626c, does not regulate the *Mycobacterium tuberculosis* growth inside the macrophage cell line, but promotes the host cell necrosis.

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In sum, Danelisvili and colleagues clearly demonstrate that the identified PPE68-Rv2626c complex has an important role in the host cell necrosis, which contributes to the *Mycobacterium tuberculosis* escape from infected macrophages. Interestingly, other members of the PE/PPE family have previously been reported to regulate Mtb pathogenesis in separate *in vivo* studies[5] and Rv2626c can modulate both innate and adaptive immune responses, suggesting its potential use as a vaccine candidate.[8] Future studies will reveal whether specific targeting of the host cell necrosis regulating factors, such as PPE68-Rv2626c, could become an efficient strategy to tackle the course of *Mycobacterium tuberculosis* infection and global tuberculosis epidemic.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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