Summary

Tuberculosis is one of the most common diseases worldwide, remaining health and social problem not only in the developing countries but also in the developed ones, including Poland. Alveolar macrophages play pivotal role in the mycobacterial immune response, since they create a first line of defense against *Mycobacterium tuberculosis* (Mtb). These cells, have developed a variety of antibacterial mechanisms that protects them from infection and allow them to eliminate pathogens. Among those mechanisms, worth mentioning are toxic factors (reactive oxygen and nitrogen species) and cytokines enhancing antibacterial properties of macrophages, as well as activating other cells of the immune system. However, Mtb have acquired numerous mechanisms, thanks to which they can avoid being killed by macrophages and can proliferate intracellularly inside phagocytes. One of these mechanisms might be DNA double-strand breaks (DSBs) repair systems. Mtb possess, specific for bacteria homologous recombination system (HR), with a RecA as a key protein, as well as non-homologous end joining system (NHEJ), where main proteins are Ku and LigD.

The main goal of this study was to evaluate the role of RecA, Ku and LigD proteins in *M. tuberculosis* defense from bactericidal properties of human macrophages, as well as against cytotoxic activities of free radicals, *in vitro*. Additionally, the functional activity of human macrophages in response to infection with Mtb with inactivated genes encoding proteins of DSBs repair systems has been evaluated.

In this research, macrophages obtained from human monocytic cell line THP-1 and/or from monocytes isolated from the buffy coat, have been used. Macrophages were infected with virulent wild-type strain of Mtb (H37Rv) or its mutants, lacking genes encoding: RecA protein (Δ*recA*), Ku and LigD [Δ(*ku,ligD*)], or all mentioned proteins [Δ(*ku,ligD,recA*)], or with complemented strains having functional copies of deleted genes encoding above mentioned proteins. What is more, dead *E.coli* bacilli have been used as a control of macrophages response to the infection. All bacteria have been opsonized or not with human serum type AB prior to macrophages infection.

The first stage of the research focused on the evaluation of HR and NHEJ systems significance in the survival of Mtb strains, *in vitro*, in the presence of nitric oxide (NO), superoxide anion ($^{\cdot}O_2^-$) or peroxynitrite anion ($ONOO^-$) donors. Results indicated that $ONOO^-$ donor, in all used concentrations, did not inhibit the growth of examined Mtb strains. On the other hand, DETA/NO (NO donor) and menadion ($^{\cdot}O_2^-$ donor) to the same extent inhibited growth and viability of wild-type strain and its mutants. This suggests that HR and

NHEJ mechanisms do not have a significant impact on the Mtb survival in the presence of NO or $^{\cdot}O_2^-$, *in vitro*.

In the following research the significance of DNA double-strand breaks repair systems in the intracellular survival of Mtb in the macrophages derived from THP-1 cell line has been assessed. Received data demonstrated that all examined Mtb strains grew similarly inside phagocytes up to 4th day after infection. On the 6th day however, it was observed that triple mutant, lacking genes coding proteins of both repair systems, grew significantly lower in comparison to the wild-type strain and other mutants. It was also noted that the ability of Δ(*ku,ligD,recA*) mutant to the similar intracellular growth as the wild-type strain was restored due to the use of proper complemented strains. Simultaneously, it was pointed that the viability of macrophages infected with wild-type strain and its triple mutant, on the 6th day after infection, was on the similar level. The inhibition of NO or $^{\cdot}O_2^-$ production in macrophages enhanced the intracellular growth of Δ(*ku,ligD,recA*) mutant up to the level of the wild-type strain, what confirms that none of these factors, *per se*, induce DNA double-strain breaks and is not able to inhibit the growth of triple mutant inside macrophages. Impaired intracellular growth of Δ(*ku,ligD,recA*) strain on the 6th day after infection was also observed in the macrophages derived from monocytes of buffy coat.

Subsequent stage of study aimed to determine the functional activity of macrophages obtained from THP-1 cell line in response to the infection with Mtb strains, lacking proteins of HR and NHEJ repair systems. The production of reactive oxygen species (ROS), NO and cytokines (TNF-α, IL-12 and IL-10) has been evaluated.

Mtb strains lacking RecA protein [Δ(*ku,ligD,recA*) and Δ*recA*] did not inhibit the production of ROS by macrophages stimulated with PMA and enhanced the production of NO by phagocytes. On the contrary, wild-type strain Mtb and Δ(*ku,ligD*) mutant significantly decreased the PMA dependent ROS production by macrophages, without inducing the NO production. The control experiments with the use of *E.coli* showed that macrophages produced significant amounts of NO and ROS after their infection with the dead *E.coli* bacilli. What is more, different functional response of macrophages to the infection of examined Mtb strains was not correlated with their ability to survive. Presented results suggest that removal of RecA protein, but not NHEJ system proteins, from Mtb, impairs their ability to inhibit the bactericidal activity of macrophages.

Evaluation of cytokine production pointed out that wild-type strain and NHEJ mutant, stimulated macrophages to TNF-α release to a greater extent than Mtb lacking RecA protein. Additionally, the level of TNF-α mRNA expression was on the similar level irrespectively of

used strain. On the other hand, there was no observed release of IL-12 by infected macrophages and mRNA expression of IL-12 p35 subunit was on the same level in phagocytes independently of used strain. As for the IL-10, macrophages infected with Δ(*ku,ligD,recA*) or Δ(*ku,ligD*) mutants released significantly lower amounts of IL-10 in comparison to macrophages infected with H37Rv strain or Δ*recA* mutant. However, the increased IL-10 mRNA expression was observed only in macrophages infected with Mtb lacking *recA* gene. Presented data suggest that Mtb without genes of DSBs repair systems, modulate macrophages' cytokines release in a various ways.

The final stage of research consisted of evaluation of MAP kinases involvement in antimycobacterial response of macrophages, as well as the assessment of examined Mtb strains impact on the level and phosphorylation of ERK1/2 proteins in phagocytes. It was observed that Mtb, lacking RecA protein, stimulate bactericidal activity of macrophages by inducing the activation of ERK1/2. The analysis of ERK1/2 protein phosphorylation showed that, in contradiction to wild-type strain, as well as Δ(*ku,ligD*) and Δ*recA* mutants, triple mutant did not inhibit ERK1/2 phosphorylation in macrophages, 24 hours after infection.

To sum up, obtained results suggest that both DNA double-strand breaks repair systems - homologous recombination and DNA non-homologous end joining are crucial to *M. tuberculosis* for their intracellular survival in macrophages. Additionally, both superoxide anion and nitric oxide, *per se*, are not sufficient for effective killing of Mtb lacking both DSBs repair systems by macrophages. It is likely that macrophages require the presence of peroxynitrite anion to eliminate Mtb lacking proteins of HR and NHEJ mechanisms. What is more, RecA protein is indispensable for Mtb to efficiently impair antibacterial functions of human macrophages *via* inhibition of ERK1/2 activity. Hence, it can be suggested that RecA protein is one of the Mtb virulence factors enhancing their intracellular survival in macrophages.