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Tuberculosis: discovery of new genes involved in the parasitism of cells by the tubercle bacillus

A European-Asian collaboration of scientists, notably including CNRS, the Institut Pasteur in Paris, the Institut Pasteur Korea in Seoul (IP Korea/Equipe Avenir Inserm¹) and the Université de Toulouse have identified ten virulence genes of the tubercle bacillus. The inactivation of these genes lessens the pathogenic **effects** of the bacillus. This discovery, published in the journal *PLoS Pathogens*, will make it possible in particular to propose original therapeutic strategies and to test novel anti-tuberculosis vaccine candidates. In order to obtain these results in only two weeks, the researchers developed a new screening technique. This innovative, rapid and efficient method could be easily transposed to other intracellular pathogens.

Despite existing medicines and BCG vaccination, tuberculosis continues to wreak havoc: every year, it kills nearly 2 million people throughout the world. This disease is caused by bacteria of the mycobacterium family, which include *Mycobacterium tuberculosis*, the agent of TB in humans. Its virulence, in other words its pathogenicity, depends on its capacity to propagate within the host cell. This pathogenic agent is in fact capable of escaping the defenses of the host, which it infects through parasitism of the macrophages, the immune system cells normally involved in the ingestion and destruction of microbes. Following inhalation, *Mycobacterium tuberculosis* enters the lungs where it is ingested by the alveolar macrophages and lies in an intracellular compartment known as a "phagosome". Normally, the function of a phagosome is to destroy the bodies ingested by the macrophage through acidification. However, instead of being killed by the cell, the tubercle bacillus multiplies within it by blocking the acidification of the phagosome. The genes of the bacillus involved in this process were poorly understood until now. Identifying them was one of the key objectives of the European-Asian collaboration coordinated by Olivier Neyrolles, CNRS researcher at the Institut de Pharmacologie et de Biologie Structurale (CNRS / Université de Toulouse) and Priscille Brodin, Inserm researcher (Institut Pasteur Korea / Inserm).

To achieve these results, the researchers firstly designed a new screening method based on the selection, by a robot, of a cellular phenotype characterized by a microscopy image. Using this innovative technique, samples

¹ The ATIP-Avenir program, jointly conducted by Inserm and CNRS, gives young high-flying researchers the opportunity to set up and head a research team for 3 years. Since their creation, these two programs have enabled **over** 438 researchers to constitute their own research teams in the life sciences and health fields.



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corresponding to the requisite criterion can be automatically and visually identified. This procedure thus makes it possible to gain considerable time in identifying the microbial entities involved in the parasitism of cells.

This novel and highly efficient method was then applied to a particularly virulent² strain of *Mycobacterium tuberculosis*. This enabled more than 11,000 mutants³ of the tubercle bacillus to be screened in just a few weeks. The robot was then programmed to detect whether the “acidification” function was active or not. This allowed the researchers to isolate the mutants unable to block acidification of the phagosome and subsequently destroyed by the macrophages. The corresponding mutations were identified by genetic engineering and ten genes, mostly unknown, involved in the parasitism of the macrophage were characterized.

Most of these genes code for the synthesis of products secreted by the bacteria: proteins and lipids, whose precise function in the parasitism of the cells by *Mycobacterium tuberculosis* still needs to be elucidated. In addition, these molecules could constitute prime targets for new antibiotics. Finally, these results suggest that the isolated mutants, the *in vivo* virulence of which is lessened in some cases, could be promising candidates in the development of new vaccines to replace the existing BCG vaccine.

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Bibliography

High content phenotypic cell-based visual screen identifies *Mycobacterium tuberculosis* acyltrehalose-containing glycolipids involved in phagosome remodeling. Priscille Brodin, Yannick Poquet, Florence Levillain, Isabelle Peguillet, Fanny Ewann, Gerald Larrouy-Maumus, Martine Gilleron, Thierry Christophe, Denis Fenistein, Jichan Jang, Mi-Seon Jang, Park Sei-Jin, Jean Rauzier, Jean-Philippe Carralot, Rachel Shrimpton, Auguste Genovesio, Jesus A. Gonzalo Asensio, Germain Puzo, Carlos Martin, Roland Brosch, Graham R. Stewart, Brigitte Gicquel and Olivier Neyrolles. *PLoS Pathogens*, 9 September 2010

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² This is the strain known as “Beijing” due to its preponderance in Asia. It is now considered as the second most on account of its global frequency and is often associated with pharmacoresistance.

³ The genome of the tubercle bacillus contains more than 4,000 genes. Scientists do not yet know the role of around half of these genes. This highlights the relevance of high-speed screening techniques: with a bank of 11,000 mutants for a genome comprising 4,000 genes, researchers are sure to have covered the whole bacillus genome.



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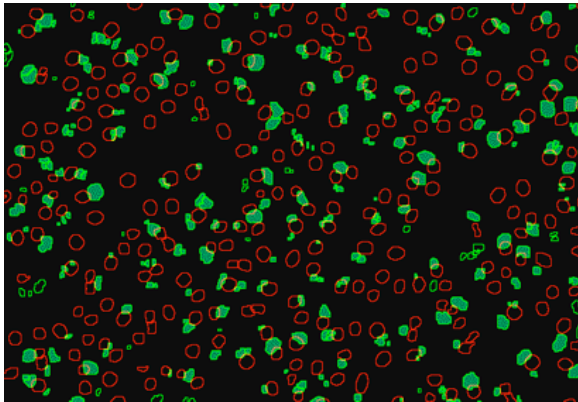


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The automatic screening method makes it possible to identify cells and bacteria as “visual objects” according to their contour: cell nuclei appear in red whereas bacteria within an acidified “phagosome” show up in green. The robot and computer then quantify the number and the surface area of the red and green “objects”, thus enabling screening.

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