Host-Pathogen Interactions in Tuberculosis

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My presentation will focus on host-cell pathogen interactions in tuberculosis. However, I would first like offer a brief introduction to the tuberculosis disease. I believe it is important to remember that merely an estimated 5-10% of individuals exposed or infected with the \textit{Mycobacterium tuberculosis} bacteria actually develop tuberculosis. In other words, the human population, as a whole, is extremely resistant to the disease. It is extremely interesting to examine the mechanisms responsible for this resistance.

However, despite this resistance, approximately 1.5 million people die of tuberculosis each year. This figure can be explained by fact that an estimated two billion people are infected with \textit{latent} tuberculosis. Thus, although the activation rates of the disease are rather low, this huge reservoir leads to high numbers of diseased and deaths.

I. Factors Influencing the Development of the TB Disease

Multiple factors explain why certain individuals are more likely to develop the disease than others.

1. Nutrition and Hygiene

The first factor that influences resistance to the disease is the quantity and quality of an individual’s nutrition and hygiene conditions. This factor is closely related to the amount of money an individual can afford to spend on his or her health. Many studies have shown, for example, that rabbits residing in dirty cages have a higher probability of developing tuberculosis than those residing in clean cages.

More recent studies describe in more detail the mechanisms by which nutrition can influence immunity and protection against tuberculosis. One study, for example, shows that a single supplementation of vitamin D can enhance immunity to the disease, at least in ex-vivo tests. A second study describes how vitamin D can improve the ability of macrophages to control infection through the production of antimicrobial peptides.

2. HIV Co-Infection, Sex and Age

A second factor influencing resistance is, of course, HIV co-infection. A third is the correlation between sex and TB. In every region of the world, between two-thirds and three-quarters of TB patients are males. Some of the reasons for this difference might be biological, although it is still a matter of debate. A fourth aspect is age, as adults are more prone to developing pulmonary tuberculosis.
3. Host Genetic Factors

There are also host genetic factors, of which merely a part has been identified. Some gene variants have been clearly associated with tuberculosis susceptibility or with protection against the disease. Again, one example is the vitamin D receptor. More recently, another study has demonstrated that promoter variation in the DC-SIGN encoding Gene CD209 is associated with increased susceptibility to tuberculosis.

4. Infecting Strain (Virulence)

A sixth factor is the infecting strain, on the bacteria side. Thanks to genotyping techniques, we are now able to discriminate *M. tuberculosis* isolates. It is now clear that any tuberculosis isolate can be clustered into different genotypes, using different techniques.

II. Public Health Measures

We currently possess two main tools to combat *M. tuberculosis*. The first is the BCG vaccine. Unfortunately, BCG is probably not sufficiently efficient to prevent TB, at least in adults. The second tool consists of four first-line antibiotics. It is noteworthy that since the 1960’s, no new first-line antibiotics has been developed. Thus, The WHO projects that by 2020, there would be 1 billion newly infected people, 200 million sick, and 35 million deaths from tuberculosis. If we wish to prevent this scenario, and live in a TB-free world, we must develop a new vaccine and new drugs to fight the disease. However, we must keep in mind that as soon as we develop a new drug, we would probably induce the emergence of new drug-resistance as well.

III. Host-Pathogen Interactions

When an individual is infected with *M. tuberculosis*, the bacilli enter his or her lungs. In order to become potentially infectious, the bacilli must first enter the alveoli. Inside the alveoli, they interact with cells, mainly macrophages and dendritic cells, through different receptors. Then, these interactions initiate the immune-response; that is, the proliferation of antigen specific lymphocytes. These lymphocytes migrate to the lungs, where they accumulate around the infected macrophages, forming a structure called the granuloma. The infected cells are located in the centre of the granuloma structure, and are surrounded by the lymphocytes.

I would like to focus on the interactions between the bacillus and the first cells it encounters in the lungs during the infection. In spite of the immune response, one of the major virulence mechanisms in TB is the ability of the bacillus to parasitize the macrophages; that is, to persist and multiply within the cells. One of the fundamental questions of tuberculosis basic research is how the bacillus can persist and multiply within these cells.

In the remainder of my talk, I would like to focus on three aspects. The first is the entry of the bacillus to the cell, and the receptors involved in this phagocytosis process. The second is the cell’s reaction to the infection. Finally, I will review the strategies used by the bacilli to persist and survive inside the cell.
1. Cell Entry

Many receptors are involved in the phagocytosis of *M. tuberculosis*. The first ones identified were the Fc receptors, involved in phagocytosis of opsonized bacteria. While it is not clear whether antibodies play a role in the protection of the host during infection, it is clear that when we opsonize mycobacteria with antibodies, and then infect the macrophage, the infection is significantly better. It is also possible to conduct opsonization with other molecules, such as complement molecules, or the Surfactant protein in the lungs. These molecules can interact with complement or Surfactant protein receptors, which mediate the internalisation of the bacteria.

It is also possible to establish a direct interaction between the bacillus and the host cell, which is mediated mostly through lectins. The best examples of such lectins are the Mannose and DC-SIGN receptors. I would like to focus on the DC-SIGN receptor, which we have been studying for the past few years.

DC-SIGN is a lectin, which means it can recognise sugar moieties. Like the Mannose receptor, it belongs to a family of C-type lectins. However, DC-SIGN and the Mannose receptors are not equivalent. Notably, DC-SIGN contains only one carbohydrate recognition site, which can bind to the sugar, whereas the Mannose receptor contains multiple carbohydrate recognition sites.

These lectins could also be differentiated according to their recognition patterns; that is, different lectins recognise different sugars. They have different endogenous ligands, and can recognise different pathogens. DC-SIGN recognises a long list of pathogens, including HIV and *M. tuberculosis*. Different lectins are also expressed by different cells types. Initially, it was believed that DC-SIGN was specific to dendritic cells. However, it seems that it is also expressed by some macrophage populations. Like some lectins, DC-SIGN is able to promote endocytosis and signalling, and to influence the host-cell response.

The first data demonstrating that DC-SIGN interacts with *M. tuberculosis* was based on a simple experiment. We incubated dendritic cells obtained from human blood, and then infected them with *M. tuberculosis*. We tried to inhibit the binding of the bacilli to the cells using different antibodies directed against DC-SIGN. The results clearly demonstrate that when the cells were incubated with antibodies directed against DC-SIGN, the binding of the bacillus to the cell was almost entirely abrogated. That phenomenon indicates that DC-SIGN is a major receptor for the bacillus in these cells.

We then examined whether DC-SIGN is involved in the interaction between the bacillus and the host cell in-vivo, in tuberculosis patients. We obtained bronchoalveolar lavage (BAL), and analysed the expression of DC-SIGN in these lavages. We used the flow cytometry technique, which measures the expression of a particular protein within the cell, and enables us to measure a particular phenotype at the single-cell level. The patients’ lungs contained two major cell populations: lymphocytes and macrophages. We have demonstrated that alveolar macrophages in TB patients express DC-SIGN at extremely high levels, from 15% to over 75%.

We were also able to demonstrate that DC-SIGN expressing cells constitute a preferential target cell population for the bacilli. As a control, we obtained lavages from patients with other pathologies, such as asthma. We have shown that very few alveolar macrophages expressed DC-SIGN in these patients.
We have identified one ligand for DC-SIGN, the lipoarabinomannan (LAM) molecule, which is exceptionally mannosylated. We conducted a simple experiment, in which we attempted to inhibit the binding of the bacteria to the cells using the LAM molecule, rather than antibodies. Our success was almost complete. Furthermore, when the mannose residues were removed, using a specific enzyme, the inhibition was entirely lost. This indicates that these particular mannose residues were critically recognised by the lectin.

Thus, the scenario we propose is that the bacillus probably interacts with a variety of receptors within the naïve host, including complement and mannose receptors. However, shortly after the infection, DC-SIGN is induced, and becomes a major receptor for the bacillus in the cells.

2. Host Cell Response to the Infection

We then wondered whether DC-SIGN, besides promoting phagocytosis, influences the immune response, and particularly the host-cell response, in other ways. The answer to this question seems to be positive. The interaction between the bacillus and different receptors on the surface of the host cells can influence the host cell response and the secretion of various cytokines. In the case of DC-SIGN, it has been shown that ligation of DC-SIGN by different mycobacterial ligands can influence the secretion of anti-inflammatory cytokines, and particularly IL-10.

A recent study examines another lectin, Dectin, which can interact with *M. tuberculosis*. Although Dectin is probably not a major receptor for phagocytosis, it might play a crucial role in the host response to the infection. The study showed that the interaction between Dectin and *M. tuberculosis* can enhance the secretion of critical cytokines in the immune response. Thus, a variety of receptors in the host cell surface can interact with the bacillus. Even if they do not mediate phagocytosis, they might have instrumental effect on the secretion of pro and anti-inflammatory cytokines, and thus play a major role in the outcome of infection in patients.

3. Intracellular Survival Strategies of the Bacillus

I would now like to focus on the strategies used by the bacillus to survive inside the cell. As I have noted, the fact that *M. tuberculosis* can survive inside macrophages is not typical. I believe that if we manage to understand how TB can parasitize the host cell, and to identify the mycobacterial genes that are involved in this process, we might be able to design new antibiotics, which are specifically directed against the products of these genes. We might also be able to generate new mutants and attenuated strains of TB, in order to develop new vaccines.

a. Understanding the TB Genome

The publication of the TB genome in 1998 by Stewart COLE and the Sanger Centre was a major milestone in the history of tuberculosis. When it was published, Douglas B. YOUNG wrote an article, in which he claimed that the entire history of tuberculosis was written in the genome, together with the clues to conquer the *M. tuberculosis* bacillus. He pointed out that it would be possible to obtain the sequence of every potential drug target and every antigen we may wish to include in a vaccine.
However, he was also clever enough to wonder whether it would be possible to convert this mass of information into a useful understanding. And indeed, the TB genome is extremely frustrating, since over 40% of its genes do not reveal any information. That is, their function cannot be deduced merely by examining their sequence.

It is therefore necessary to seek other methods to understand the TB genome. One approach is to compare different genomes. For example, we can compare pathogenic and non-pathogenic bacteria. It is also possible to compare the products of the genomes, rather than the genomes themselves. For example, comparing the transcriptomes and proteomes in different conditions might help us deduce useful information.

b. Functional Genomics

Finally, it is possible to conduct functional genomics. Under this approach, we mutate the genomes, rather than merely compare them. We then screen the mutants, in order to assess the function of different genes, and obtain gene candidates for drug design.

An example of such technique is the Signature tagged Transposon Mutagenesis (STM), which enabled the identification of the first virulent gene cluster in *M. tuberculosis*. The technique was initially developed for Salmonella, and then adapted for many pathogens, including *M. tuberculosis*. The basis of the technique is the creation of a library of mutants using a transposon. These mutants are then screened using a particular probe, which is located inside the transposon. It is therefore possible to track the mutants during the infection process, using merely PCR and hybridisation.

The technique has been used to infect mice with a collection of mutants. A few weeks following the infection, the lungs of the mice were extracted, the bacteria were recovered, and the DNA was obtained. Then, the composition of the mutant pools was analysed again. If a mutant disappeared following the screening process, it indicated that it was not happy inside the mouse. The next step was to identify the transposon insertion site, using molecular biology techniques, in order to know in what gene the transposon is inserted. Even if the gene had no annotation, we could at least know that the gene plays an important role in the bacteria infection of the mouse. The STM technique also allowed to identify a large cluster in the TB genome, which encodes large lipids in the cell envelope of *M. tuberculosis*. These lipids play a critical role in mycobacterial virulence.

Similar techniques can be used to screen mutant libraries not merely in mice, but also in cells. Since we use a larger collection of mutants, we do not use hybridisation anymore, but real-time PCR. Following one week of infection, it is possible to identify genes that are relevant for the particular screening process, and cluster them into major families. Besides lipids, we also found proteins involved in bacterial metabolism and respiration. It is noteworthy that we have also identified genes with fully unknown functions. We have then discovered that some of these genes are critical for the infection process within the host cell. While we must still discover the function of these genes, at least we obtained some candidates.
c. Functional Genomics at the Phagosome Level

I would like to discuss functional genomics at the phagosome level, and not merely the level of the entire organism or host cell. The first paper on the cell biology of *M. tuberculosis* infection has been published in 1975 by ARMSTRONG and D’ARCY HART. The paper examined the trafficking of the bacillus within the macrophage, and I believe is an historical paper. It is particularly striking that the questions posed by the paper’s authors 30 years ago, are the same questions we deal with today.

They discovered that the *M. tuberculosis* bacillus can persist and multiply inside host cells because it is able to block the maturation of the vacuole in which it resides, which is called the phagosome. When a particle is phagocytosed by a macrophage, it is located inside a vacuole that progressively fuses with the lysosomes inside the cell. However, in the TB case, the phagosome does not fully fuse with the lysosome.

Today, we believe that the TB bacillus resides in a vacuole that is mildly acidic. Typically, the pH of a fully mature phagolysosome is around 5. However, the pH of a mycobacterial phagosome is around 6.5 or above. In addition, the vacuole does not accumulate the V-ATPase protein, which is responsible for pumping the protons from the cytoplasm to the vacuole. It does not fuse with the lysosomes, and maintains the characteristics and markers of early endosomes. Thus, the phagosome is accessible to the external part of the cell, through recycling endosomes.

Several bacterial factors were shown to be involved in the process of blocking phagosome-lysosome fusion. These factors include PknG, LAM, and SapM. For example, it has been shown that LAM and SapM can inhibit the production of PI3P, which is critical in the phagosome-lysosome fusion. Thus, the SapM phosphatase, produced by the bacillus, can dephosphorylate PI3P, which is one way by which the bacillus can prevent the fusion. However, the inhibition is not complete, but merely 30-40%, indicating that SapM is not the only factor involved in the blockade process. Thus, multiple mechanisms are involved in the process of inhibiting phagosome-lysosome fusion.

I believe we must develop new methods to screen TB mutant libraries not merely at the organism or host cell level, but at the host cell intracellular compartment level. Two impressive papers were recently published on this topic, from the laboratories of David RUSSELL (2004), Graham STEWART (2005) and Douglas YOUNG (2005). In conclusion, I hope I succeeded in convincing you that using functional genomics might help us propose new targets for promising antibiotics and vaccines in the coming years.

IV. Question and Answer Session

Participant

Do the mycobacteria actively block the acidification of the vacuole?

Olivier NEYROLLES

That is not clear. The first paper, from David RUSSELL’s laboratory, reveals that the mycobacterial phagosome does not accumulate the V-ATPase protein. This protein is critical in the acidification of vacuoles, since it pumps the protons inside. Whether M. tuberculosis has a particular mechanism to actively exclude the V-ATPase from the phagosome membrane
is not clear yet. There are also probably some factors secreted by the bacteria that influence the pH inside the vacuole.

Participant

Is it possible to eradicate TB at the intracellular level?

Olivier NEYROLLES

In vitro, we have discovered several elements that can help fight the bacillus. For example, if we activate the macrophage using particular cytokines, we can help the cells control the infection. If we activate the cells with interferon-gamma, for example, it is possible to make the cells produce radicals that are toxic to the bacillus. However, I do not believe it is possible to eradicate the bacteria completely using merely biological compounds, such as cytokines or vitamins.

Participant

Have you used DC-SIGN as a pharmacological target? Perhaps blocking DC-SIGN could help block TB infection.

Olivier NEYROLLES

I do not believe it is possible. In any case, the bacillus does not use DC-SIGN exclusively to enter the cell, so it would find different ways to do so.

Participant

Can DC-SIGN influence the immune-response?

Olivier NEYROLLES

It might, if it influences the signalling. In fact, we found SNPs in the DC-SIGN promoter associated with susceptibility to TB, indicating that the level of DC-SIGN expression influences the disease. However, I would not go as far as to suggest that we could manipulate DC-SIGN to help patients.

Ying ZHANG

What is the influence of DC-SIGN on the survival of the bacteria within the cell?

Olivier NEYROLLES

The consensus today is that entry through the complement receptor or the mannose receptor is quite safe for the bacteria. Unpublished experiments from the lab indicate that entry through DC-SIGN probably does not influence the survival of the bacteria inside the cell.