

# Artigo de Revisão

## The resurgence of tuberculosis and the impact of the study of pulmonary immunopathogenesis\*

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The resurgence of tuberculosis as one of the most important infectious diseases to affect mankind came after the illusion that the disease was under control and would be eradicated before the end of the 20<sup>th</sup> Century. Over the last 10 years, in association with American and European research centers, our group at the Universidade Federal do Rio de Janeiro has been dedicated to investigating the pathogenic mechanisms involved in pulmonary tuberculosis. Due to its frequency and role in transmission, pulmonary tuberculosis is the most serious form of the disease. Our hypothesis is that the establishment of latent infection and its progression to active disease depend on an imbalance between activating and deactivating cytokines at the disease site. Despite the presence of protective mechanisms such as the macrophage expression of phenotypes (denoting cellular and molecular activation of agents involved in protection, such as nitric oxide and interferon- $\gamma$ ), tuberculosis progresses. A possible explanation for this is the concomitant presence at the site of infection of molecules such as interleukin-10 and TGF- $\beta$ , which are able to deactivate previously activated macrophages. Recent data suggest that mycobacteria secrete proteins capable of inducing interleukin-10, thus contributing to overcoming host protective mechanisms. Susceptible individuals would be more able to produce larger amounts of these molecules due to genetic polymorphisms that facilitate interleukin-10 production at infection onset. The understanding of these mechanisms could advance the prevention and discovery of new therapeutic targets for the control of tuberculosis.

**Key words:** Tuberculosis/ethiology. Tuberculosis pulmonary/pathology.

Study carried out at the Universidade Federal do Rio de Janeiro (Rio de Janeiro Federal University) and Weill Cornell Medical College, Université de Paris VI (VI Paris University)\*\*.

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## INTRODUCTION

The phrase “the resurgence of...” used in our title is quite significant if we consider it as a marketing ploy. Those who are familiar with the history of tuberculosis (TB) in Brazil will recognize it as an untruth. In actuality, TB has never ceased to be a problem in our country, or in third-world countries in general. What did change was the prevalence of TB in developed countries. It was believed that TB had been virtually eradicated in the so-called first-world countries. Prominent authors stated that TB was being eradicated worldwide, just as smallpox had been. However, they were actually referring to the eradication of TB in first-world countries. In the 1970s, the prevalence rates of TB in Holland were extremely low and were falling even lower. The same was true in the rest of Europe and in the United States. In other words, first-world countries were actually making progress in the eradication of TB. What has changed? What has made TB a disease that is no longer considered eradicable, at least in the beginning of this new century? The year 1985 represents the moment at which TB indices began to rise once again. This is attributable to a number of factors, one of which is increased migration. Reservoirs of TB exist in Africa, South America and Central America. Asia is one of the great reservoirs of TB in the world, rivaled only by India in the statistics. Considering that international migration continues to increase, especially from these reservoir areas to developed countries, it is obvious that these reservoirs will eventually be replicated in first-world countries, despite TB control programs in those developed countries that open their doors to immigration.

Nevertheless, there is one historical factor that can be held responsible for the changes in the TB prevalence curve: the acquired immunodeficiency syndrome (AIDS) pandemic, which introduced a new reality, since individuals infected with TB who also become infected with the AIDS virus rapidly convert to active TB. Additionally, TB does not require a vector for its transmission, being transmitted from person-to-person. A human immunodeficiency virus-positive (HIV+) individual is at greater risk for developing TB. When the active form of the disease is attained, the bacillus may be transmitted to individuals who are not HIV+, such as health professionals, prison mates and

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### Abbreviations used in this paper:

AIDS	- Acquired immunodeficiency syndrome
HIV	- Human immunodeficiency virus
HLA-DR	- Human leukocyte antigen-D region
Mtb	- Mycobacterium tuberculosis
NOS2	- Inducible nitric oxide synthase
TB	- Tuberculosis
TGF	- Tumor growth factor

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shelter mates. Therefore, the prevalence of TB increases exponentially, resulting from a situation that is not directly or necessarily related to the AIDS virus. This is why TB has again been in the headlines and is considered to be on the rise, which led the World Health Organization to declare TB a global health emergency in 1995.

We must bear in mind that we are referring to a disease that has afflicted humankind for ages. We know that TB has been found in material taken from 4000-year-old Egyptian mummies, in which it was identified using molecular biology techniques that employ known sequences of the genome of these mycobacteria. Evidence of TB has also been found in the bones of Neolithic humans. In the Middle Ages, it was called the “white plague”. There is a renewed interest in TB exhibited by the media, which could result in additional funding for research. In fact, lack of financial support for research is the primary reason that our understanding of TB has not kept pace with the advance of the disease. Therefore, we view TB as it was in the last century. In clinical practice, we still use techniques that have been used for up to 100 years. Many of our diagnostic methods, such as purified protein derivative testing, were developed 100 years ago. Our therapeutic regimens, including anti-TB drugs such as isoniazid and streptomycin, were developed 50 years ago. In addition, the vaccines currently in use, such as the bacillus Calmette-Guérin vaccine (which was developed in 1909) are quite dated. Investment in TB research pales in comparison to the solid financial support provided for research into other diseases such as AIDS.

Tuberculosis has always been a problem in Brazil, although the historical and current lack of funding for TB research has perpetuated this problem. In first-world countries, which traditionally invest heavily in research in the broader areas of

Biology and Biomedicine, TB has effectively been left behind. This is a disease that will kill 30 million people worldwide over the next ten years. There are few comparable situations. When we consider the level of financial support for TB research and TB-control programs worldwide, it becomes obvious that the sum invested is totally inadequate and disproportionate. Priority should be given to TB, although, unfortunately, there are precious few who think so.

Even prior to the introduction of chemotherapy, epidemiological indices of TB were falling, especially in first-world countries. At the beginning of the 20th century, there was a high prevalence of TB in the United States, where progressive improvement in quality of life eventually brought about a significant reduction in all epidemiological indicators, even prior to the introduction of specific chemotherapy. The introduction of streptomycin, the first effective anti-TB drug, resulted in a second considerable reduction in TB indices, which was repeated years later, when isoniazid was introduced. The understanding of the postulations of chemotherapy dates from the same period, indicating that the use of these drugs should be used in combination in order to avoid the appearance of resistant strains. From the beginning of the 1950s to the beginning of the 1980s, there was an enormous reduction in the incidence of TB in the USA, in Europe and in the rest of the developed world. However, TB incidence was also reduced significantly in poor countries such as Brazil and India. This was due to an overall global improvement in sanitation, even in poorer countries. General development, especially in the areas of technology, science and sanitation over the course of the century facilitated the control of all diseases. This phenomenon is not restricted to TB alone, although its control also benefited from the general improvement in quality of life. However, midway through the 1980s, there was an inflection in the TB prevalence curve, resulting in an ascendant tendency, which still prevails.

### The pathogeny of tuberculosis

The immunopathogeny of TB, or rather the way the host immune system acts to impede the progress of the disease, is a subject that has suffered significantly from the lack of investment in research. Among the TB-related facts mentioned

above, one in particular calls attention to the need for research in this area: TB has infected one-third of the global population, in which there are approximately 10 million cases of active-TB. Because they possess defense mechanisms that keep the mycobacteria in check, thereby impeding its activity, the remaining infected individuals do not develop the disease. Obviously, if we can gain a reasonable understanding of this defense mechanism, we will be able to comprehend the other side of the equation: what causes 5% of all contaminated individuals to develop the disease.

Infection with *Mycobacterium tuberculosis* (Mtb) has three possible outcomes: resolution at the point of entry (due to innate immunity), active disease or latent TB. In the last case, the organism controls, but does not eliminate, the infection. The Mtb remains dormant, replicating intermittently and presenting an altered metabolism. This creates a massive reservoir of TB. Several questions are relevant to this problem. Which initial events lead to immediate control of the infection? Which factors contribute to the onset of latent infection? By identifying the mechanisms involved, we can propose vaccinations, chemotherapies and adjuvant immunotherapies that may prevent patients from developing the active form of TB.

Immunology effectively began with the discoveries made by Robert Koch in the 1890s. However, it may be considered a very recent science, since the T lymphocyte and the B lymphocyte were not identified until the beginning of the 1970s. Even then, we knew nothing about cytokines, the understanding of which has brought about a revolution in the field of biology.

The organism defends itself against the mycobacteria with the aid of two cell types: the T lymphocyte and the macrophage. When the mycobacteria invade the lung of the subject after having been expectorated in microparticles by a tuberculous patient, it is initially phagocytosed by an alveolar macrophage at the first close contact with the lung. Depending on the virulence of the bacillus and the quantity inhaled, this macrophage may or may not resolve the problem there, at the point of entry. However, the macrophage is rarely capable of destroying the mycobacteria alone. It needs the assistance of other cells, especially in the form of cytokine production, which will enhance the ability of the macrophage to kill the

mycobacteria within its own cytoplasm. In addition to producing important cytokines, T lymphocytes, in their effector role, represent a powerful weapon, since they are able to destroy macrophages that have succumbed to the mycobacteria. Once the T lymphocyte recognizes that the macrophage has lost this battle, it kills the macrophage, releasing the bacillus into the environment, where more effective macrophages can phagocytose and control it. This represents an effector action of the T lymphocyte itself against mycobacteria, albeit in an indirect way.

We have begun to recognize certain cell subpopulations, such as the T  $\gamma\delta$  lymphocytes, which play a very important role in combating TB since they are highly toxic to infected macrophages and produce cytokines that stimulate the remaining macrophages to kill the mycobacteria. Even the macrophage is no longer simply considered an effector cell, but also one of the most important inducers of cytokine production. In light of this new knowledge, we moved away from the concept that induction of the immune response would always be carried out by T lymphocytes and that the effector action would always be provided by the macrophage. We now know that the T lymphocyte and the macrophage may play the roles of both inductors and effectors.

Another reason for reevaluating our previous conception of TB is the advent of HIV. The AIDS pandemic has imposed on humankind a revolution in the field of immunology. Never before has there been progress such as that seen in the last 20 years. An HIV+ patient presents a form of TB that is very different from that seen in immunocompetent individuals: it is much more severe, more often fatal and has a much worse effect on the survival of the patient. Individuals die more rapidly when suffering from TB and HIV concomitantly. Even if the TB is cured, AIDS survival time is reduced<sup>(1)</sup>.

### **The role of cytokines in the development of tuberculosis**

It is essential to understand the mycobactericidal mechanisms of macrophages and how the immune system, especially the pulmonary immune system, participates in these mechanisms. In virtually all cases, TB transmission occurs via the airways, and 80% present as the pulmonary form of the disease.

In addition, the pulmonary form is the only form that is epidemiologically significant, since it allows person-to-person transmission, thereby maintaining the disease transmission cycle. Cytokines are hormones that are the products of several types of cells within the organism and have a variety of functions. In the case of TB, specifically, they play very important roles, which we are only beginning to understand.

We understand that, to be protected against TB, an individual needs to possess a particular type of cytokine that enables macrophages to kill the mycobacteria. However, we are also beginning to understand that there are certain types of cytokines that, inversely, deactivate macrophages, thereby preventing the destruction of the mycobacteria. There are cytokines of both Th1 and Th2 profiles. In short, the Th1 cytokines are those, such as IFN- $\gamma$  and IL-2, which activate the macrophage microbicidal mechanisms, and Th2 cytokines, such as IL-4, IL-5 and IL-10, deactivate the macrophage. Depending on the prevalence of these types of cytokines, TB will progress or will be prevented. Our hypothesis is that there is a prevalence of deactivating cytokines when the disease develops, even when activating cytokines are present. If the infected individual has a genetic tendency to produce a greater amount of IL-10, for example, his chances of developing TB will be higher. Therefore, genetic polymorphisms, which facilitate a greater production of IL-10 when facing an infectious disease, within certain populations may be used in the future as genetic markers for the study of groups with higher or lower risk of developing active TB. There are ways to identify population groups which are high or low producers of IL-10. If an individual contaminated by mycobacteria is a major producer of IL-10, he will be treated with greater care, and will probably be required to take an IL-10 inhibitor.

As yet unpublished studies that were recently conducted by our group, led by Dr. Adalberto Rezende, have shown a statistically significant correlation between certain haplotypes that are facilitators of IL-10 production and higher rates of active TB. In an experimental study in mice that have been genetically altered in order to present exaggerated expression of IL-10, it was shown that normally sublethal doses of mycobacteria are fatal for these animals<sup>(2)</sup>. In contrast, the authors

demonstrated that wild-type mice, that produce a lower amount of IL-10, survive the same dose levels, showing that exaggerated production of IL-10 reduces the chance of surviving a mycobacterial infection.

Our group at the *Universidade Federal do Rio de Janeiro* (Rio de Janeiro Federal University) has worked in conjunction with Cornell University in New York for the last ten years, developing and publishing various studies that focus on evaluating cells collected from TB-affected areas of the lungs. Our aim has been to determine whether there is enhanced or reduced expression of IL-10 or other cytokines with similar actions that could generate this immunological dysfunction. Our studies included patients with pulmonary TB submitted to diagnostic bronchoalveolar lavage because they were not producing sputum or produced sputum that tested negative for mycobacteria. The technique involves drawing and centrifuging the fluid, after which the supernatant is stored and the cells are studied. In order to determine whether there is exaggerated expression of certain cytokines, the whole cell may be studied in the cytocentrifuged material, or the cell can be flattened and the genetic material extracted. In one of the early studies<sup>(3)</sup>, we determined the phenotype of the T lymphocytes and macrophages, and we found that TB patients with concomitant HIV also presented dysfunction within the macrophage population. In general, we believe that, in HIV infection, there are basic defects in the T CD4+ lymphocyte, whereas, in patients with TB and HIV, the macrophage itself becomes unable to generate a defense against the mycobacteria. Epithelioid cells identified by specific monoclonal antibody are present in significantly greater numbers in the bronchoalveolar lavage fluid of immunocompetent patients, whereas their numbers are reduced in HIV+ patients. We also studied the macrophage expression of certain molecules, such as the human leukocyte antigen-D region (HLA-DR), which is a T lymphocyte antigen-presenting cell. When this molecule is found in great quantities, it results in enhanced antigen-presenting ability. In TB patients, the quantity of HLA-DR is much greater than in controls, whereas in TB patients with concomitant HIV it is very much diminished, demonstrating a defect within the macrophage itself.

We also studied nitric oxide, which is another molecule that is very important in the defense against mycobacteria, and the nitric oxide inducer enzyme, also known as inducible nitric oxide synthase (NOS2). We demonstrated increased enzyme expression<sup>(4)</sup> within the population of patients with TB. Therefore, although we have demonstrated that there is an increase of HLA-DR, as well as an increase in the NOS2 enzyme (both of which should, theoretically, protect these individuals), they continue to be ill. We believe this is because, even in the presence of increased expression of these defense factors, something is impeding the protection. We hypothesize that the deactivating cytokines are responsible for this hindrance. In a subsequent study, we assessed the quantity of cytokines present in the bronchoalveolar lavage fluid of TB patients<sup>(5)</sup>. We determined that levels of the cytokine that activates macrophage microbicidal function, namely IFN- $\gamma$ , are increased, as we want them to be. However, the patient presents active TB and will die if not treated, demonstrating that IFN- $\gamma$  alone has no effect. This is probably due to the fact that, within the same material, there is coexpression of deactivating cytokines, which perform a significant physiological function in containing the immune reactions after the stimulus for that reaction has been withdrawn. These are anti-inflammatory cytokines, which are important in interrupting the inflammatory process. The problem is that their expression is apparently increased when it should not be. Recent studies conducted by our group have shown this coexpression of activating and deactivating cytokines. Furthermore, another deactivating cytokine known as tumor growth factor- $\beta$  (TGF- $\beta$ ) is also increased. In patients with TB, we have demonstrated that, in addition to increased expression of this cytokine, numbers of the cellular receptors required for TGF- $\beta$  activity are also increased<sup>(6)</sup>.

This imbalance aids the establishment and progress of the infection. As previously mentioned, there are genetic alterations that could facilitate the exaggerated production of cytokines such as IL-10 at a moment when their presence at the site of infection may cause the immune protector response to become completely unbalanced. There is increasing evidence that, in its growth phase, *Mtb* itself secretes some proteins that are capable



of significantly interfering with the immune response – working in its favor, so to speak. Recently published studies conducted by our group at the University of Cornell show that one of these proteins, known as CFP32, is expressed only by members of the Mtb complex and is not expressed by other species of mycobacteria. *In vitro* and *ex vivo* studies have shown that this protein is able to induce considerable production of IL-10, which, as previously mentioned, facilitates the progress of the infection<sup>(7)</sup>. The presence of this gene in the mycobacteria has advanced the development of new diagnostic methods as well as aiding the identification of subspecies within the genus, with implications for studies that focus on molecular epidemiology<sup>(8)</sup>.

### Role of dendritic cells

Another cell type that merits further study, due to its ability to induce a potentially long-lasting protective immune response to TB, is the dendritic cell. Sufficient numbers of this type of cell, together with other types of cells of the monocyte-macrophage lineage, are essential for granuloma formation, which simultaneously protects the host from dissemination of the infection and protects the microorganism from being eliminated<sup>(9)</sup>. A dense cellular population and the presence of certain cytokines, such as type-I interferon, are essential for granuloma formation and reduction of the bacterial load<sup>(10)</sup>. Recent studies conducted by our group, in association with researchers from the *Institut Pasteur de Paris* (Paris Pasteur Institute) have shown the importance of these mechanisms in establishing a protective response to TB<sup>(11,12)</sup>.

In conclusion, the study of the mechanisms through which the host defends itself against Mtb infection, as well as of the potential mechanisms by which the mycobacteria manipulates the host immune system in its own favor, may shed new light on the pathogeny of TB. The result of this would be better vaccines, new therapeutic targets and more precise diagnostic methods for combating this terrible illness.

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