The resumption of consumption – A review on tuberculosis

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Among all infectious diseases that afflict humans, tuberculosis (TB) remains the deadliest. At present, epidemiologists estimate that one-third of the world population is infected with tubercle bacilli, which is responsible for 8 to 10 million new cases of TB and 3 million deaths annually throughout the world. Approximately 95% of new cases and 98% of deaths occur in developing nations, generally due to the few resources available to ensure proper treatment and where human immunodeficiency virus (HIV) infections are common. In 1882, Dr Robert Koch identified an acid-fast bacterium, Mycobacterium tuberculosis*, as the causative agent of TB. Thirty-nine years later, BCG vaccine was introduced for human use, and became the most widely used prophylactic strategy to fight TB in the world. The discovery of the properties of first-line antimycobacterial drugs in the past century yielded effective chemotherapies, which considerably decreased TB mortality rates worldwide. The later introduction of some additional drugs to the arsenal used to treat TB seemed to provide an adequate number of effective antimicrobial agents. The modern, standard short-course therapy for TB recommended by the World Health Organization is based on a four-drug regimen that must be strictly followed to prevent drug resistance acquisition, and relies on direct observation of patient compliance to ensure effective treatment. Mycobacteria show a high degree of intrinsic resistance to most antibiotics and chemotherapeutic agents due to the low permeability of its cell wall. Nevertheless, the cell wall barrier alone cannot produce significant levels of drug resistance.* M. tuberculosis *mutants resistant to any single drug are naturally present in any large bacterial population, irrespective of exposure to drugs. The frequency of mutants resistant to rifampicin and isoniazid, the two principal antimycobacterial drugs currently in use, is relatively high and, therefore, the large extra-cellular population of actively metabolizing and rapidly growing tubercle bacilli in cavitary lesions will contain organisms which are resistant to a single drug. Consequently, monotherapy or improperly administered two-drug therapies will select for drug-resistant mutants that may lead to drug resistance in the entire bacterial population. Thereby, despite the availability of effective chemotherapy and the moderately protective vaccine, new anti-TB agents are urgently needed to decrease the global incidence of TB. The resumption of TB, mainly caused by the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains and HIV epidemics, led to an increased need to understand the molecular mechanisms of drug action and drug resistance, which should provide significant insight into the development of newer compounds. The latter should be effective to combat both drug-susceptible and MDR/XDR-TB.*

Key words: *Mycobacterium tuberculosis* - epidemiology - chemotherapy - vaccine - drug action - mechanism of resistance

There is a dread disease which so prepares its victim, as it were, for death; which so refines it of its grosser aspect, and throws around familiar looks, unearthly indications of the coming change – dread disease, in which the struggle between soul and body is so gradual, quiet, and solemn, and the result so sure, that day by day, and grain by grain, the mortal part wastes and withers away, so that the spirit grows light and sanguine with its lightening load, and, feeling immortality at hand, deems it but a new term of mortal life; a disease in which death takes the glow and hue of life, and life the gaunt and grisly form of death; a disease which medicine never cured, wealth warded off, or poverty could boast exemption from; which sometimes moves in giant strides, and sometimes at tardy pace; but, slow or quick, is ever sure and certain.

Charles Dickens, 1870, in Nicholas Nickleby, Wiendenfeld and Nicholson, London, p. 243.

Human tuberculosis (TB) is a contagious-infectious disease mainly caused by *Mycobacterium tuberculosis*, which is an aerobic pathogenic bacterium that establishes its infection usually in the lungs. Progression of TB infection is fundamentally regulated by host's immune system integrity, which may succeed through microbial immediate elimination and/or latency conditioning, or fail resulting in development of active disease.

TB was responsible for millions of human deaths in the past, when there were no adequate treatment methods for infected patients. Introduction of chemotherapy and prophylactic measures led to drastic death reduction, which was maintained for various decades. However, the "good times" waned, as this disease became worldwide recognized as the one responsible for most human deaths caused by a single infectious agent. TB resumption is basically a consequence of anthropic factors, such as the recent HIV/AIDS pandemic and the development of drug-resistant strains (stemmed from inappropriate treatments and/or patient non-compliance). It thus appears to be of fundamental importance to increase investment in research, as disease control can hopefully be reached

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through new drug development (to be introduced in the treatment of patients with active TB), and through prophylactic and/or therapeutic vaccine optimization.

Looking the past

The captain of all these men of death that came against him to take him away, was the consumption, for it was that brought him down to the grave.

John Bunyan, 1680, in The Life and Death of Mr. Badman, Dent, London, 1928, p. 282.

TB, also known as the white plague, received the title of "captain of all these men of death" by John Bunyan in the second half of the XVII century, when the disease reached a high level of death rates in Europe. This malady became the principal cause of death by the end of the XIX and beginning of the XX century, and among its various victims were worldwide known people, such as Frédéric Chopin, Paganini, St. Francis of Assisi, Charlotte Brontë (and most of the Brontë family), John Keats, Lord Byron, George Orwell, Castro Alves, Alvarez de Azevedo, Cruz e Souza, Augusto dos Anjos, Noel Rosa, Eleanor Roosevelt, and Vivian Leigh, among many others. Currently, this disease still represents a global threat, as it stands as the leading cause of death due to an infectious agent among adults worldwide.

Although it was probably described for the first time in Indian texts, pulmonary TB is known since the time of Hippocrates as phthisis, which is derived from the Greek for "wasting away". Scrofula, a rare manifestation form of TB that affects the lymph nodes, especially of the neck, most commonly found in children and usually spread by unpasteurized milk from infected cows, was well documented during the European Middle-Age, when it was believed that cure resulted from the power of the divine touch of the kings. Pott's disease or Gibbous deformity, a rare TB manifestation, revealed only among several antique Egyptian mummies, is a destructive form of TB that leads to serious spine deformities and subsequent member paralysis.

In 1680, the French Franciscus Sylvius carried out anatomic-pathologic studies in pulmonary nodules from TB patients, which he named as "tubercula" (small knots), observing their evolution to lung ulcers (cavities). However, most of the great pathologists of his time believed these knots were some type of tumor or abnormal gland, rejecting any probable infectious origin. The first credible speculation of the infectious nature of TB was performed by the British Doctor Benjamin Marten, who proposed in 1722 that TB could be transmitted through the "breath" of a sick person, inhaled by a sound one, and thereby turning her ill. In 1689, the English Doctor Richard Morton used the term "consumption" to specifically denote TB, and finally, in 1819, the inventor of the stethoscope, the French Doctor René Laennec identified for the first time the TB manifestation unit.

As the disease became completely established among every European social level, afflicting many of the intellectual and artists of the continent by the half of the XIX century, TB was romanticized, as typical symptoms like thin and pale faces of the infected ones became signs of beauty. The romantic Era of TB can also be recognized in fine pieces of art, such as in the famous painting of William Morris, which exhibits all the splendor of the legendary Guinevere, King Arthur's wife, already displaying typical symptoms of the active disease.

In 1865, the Military Surgeon Jean-Antoine Villemin demonstrated formally that TB is a contagious disease; although his experimentation could be effectively reproduced in rabbits, the finding was ignored by his contemporaries for a long time.

One of the greatest works on TB was performed in 1882 by Robert Koch, an esteemed scientist of his time. Koch isolated and cultured *M. tuberculosis* from crushed tubercles. His experimental work identified the bacterium as the TB etiological agent (Bloom & Murray 1992, Daniel 1997). In August of 1890, during The First Ordinary Session of the International Medical Congress, in Berlin, he announced the discovery of a TB therapeutic drug. Three months later, the "Deutsche Medizinische Wochenschkift", in extraordinary edition, published a new statement of Koch, revealing that although interested in the therapeutic properties of his findings, he observed that the referred liquid, named tuberculin, could be useful as a diagnostic tool to detect the disease due to the intensified reaction developed by sick animals inoculated with this drug, as no measurable effect was ever observed in healthy ones. This concept was perpetuated for several years, until it was observed that even healthy animals could react to the drug. The veterinarians of his time clarified the fact by demonstrating that the healthy ones could be simply infected, although not ill. As a result, it was established that *M. tuberculosis*-infected animals will react to tuberculin infusion, whereas the non-infected ones will not. This drug, the first industrialized one, was called old tuberculin; subsequently, other tuberculins were produced, such as purified protein derivate (PPD), PPD-S, and PPD RT23, among others (Vaccarezza 1965, Ruffino-Netto 1970). The tuberculin skin test became the principal tool for infection diagnosis. In the same period, Koch developed staining methods for the identification of the bacillus; these techniques were subsequently improved by the German Doctor and bacteriologist Paul Ehrlich, whose method for detection of the bacillus provided the basis for the development of the Ziehl-Nielsen staining, which still is an important tool to diagnose TB.

Koch's discovery allowed researchers to focus efforts on the development of new and more efficient therapies to treat TB patients. One of the first attempts to fight the disease was performed by Edward Livingston Trudeau, who suffered from TB and was subsequently cured. Trudeau established the first sanatorium in the United States in 1884. This institution received only TB patients, and invested in a treatment based on rest, fresh air and a healthy diet.

In 1896, the American bacteriologist Theobald Smith demonstrated that bovine TB was not caused by *M. tuberculosis*, but rather by another species, *M. bovis*. Twelve years later, the scientist-couple Albert Calmette and Camille Guérin isolated the bovine variant from its host and grew the bacilli in dispersed culture containing ox bile. By the 39th passage they observed a morphological variant that was avirulent in several animal models and

which conferred immunological protection against subsequent challenges with virulent *M. tuberculosis*. Thirteen years of experimentation led to the obtaining of the 231st passage, the variant that was administered for the first time in humans (orally), as an attempt to immunize a child whose mother died in childbirth victim of TB. Currently known as BCG (bacille Calmette-Guérin), the (intradermal) vaccine has become widely used to combat TB; it relies on a prophylactic administration of live attenuated bacilli to children.

The introduction of antibiotics, such as streptomycin (1947), isoniazid (synthesized in 1912, but introduced 40 years later) and *p*-aminosalicylic acid, led to a TB chemotherapy revolution, as TB mortality rates were considerably reduced (Bloom & Murray 1992, Daniel 1997). Subsequently, other anti-TB drugs were also developed, such as ethambutol and rifampicin, among others. Since the mid 1980s, however, there has been no new first-line drug development to fight the TB causing bacilli (Petrini & Hoffner 1999).

In Brazil, it is believed that the disease was introduced by the Portuguese and Jesuit missionaries since 1500. Oral BCG was administered for the first time by Arlindo de Assis in 1927 to newborn, and intradermal vaccination was implemented in 1973, becoming obligatory for one year minors since 1976. Brazilian TB mortality rates were drastically reduced due to introduction of tuberculostatic drugs by the 1940s, including streptomycin (1948), *p*-aminosalicylic acid (1949), and isoniazid (1952). The standard chemotherapy treatment recommended by the World Health Organization (WHO) to control or eradicate TB worldwide, which is based on a short-course therapy that combines the use of four anti-TB drugs, currently known as directly observed treatment short-course (DOTS), seems to be used in Brazil since 1962 by the Fundação de Serviço Especial de Saúde Pública (Sesp) in units of all complexity levels (Ruffino-Netto 2002).

Tuberculosis resumption

In 1993 the WHO declared TB a global public health emergency, being the only disease so far to warrant that designation. Although hospitals have been established and chemotherapy has been developed to combat TB, which have brought considerable reduction in incidence to developed nations, historical data calculated by the WHO indicate that there have not been great effects on the global problem since the time of Koch (Bloom & Murray 1992). Currently, TB is responsible for more human deaths than any other single infectious agent, standing for 26% of all preventable deaths and 7% of all deaths (Enarson & Murray 1996).

TB resumption has been attributed to several factors, such as the increase in drug resistance; the HIV/AIDS pandemic (in the beginning of the 1980s); the increase of injectable drug users; changes in social structure; the increase of immigrants from high prevalence nations to developed ones; the aging of the world population; the active transmission amongst environments of human accumulation (such as prisons, hospitals, and homeless shelters); and the degradation of health care systems (Fätkenheuer et al. 1999). Although TB became a reemerging disease to European and North-American nations, TB is not an emergent nor reemerging public health problem in developing countries such as Brazil, but rather a long lasting one (Ruffino-Netto 2002).

In order to facilitate the comprehension of the various components involved in the interaction between these factors, Ruffino-Netto (2004) proposed an "equation" which expresses the TB charge, represented as follows:

$$\text{TbB} \approx \frac{(\text{SIN}).(\text{PHIV}).(\text{PDEF}).(\text{PR}).(\text{MIG}).(\text{OLDP})}{(\text{AHS}).(\text{DOTS}).(\text{EDU}).(\text{NUT}).(\text{HRTb}).(\text{DPP})}$$

where SIN: social inequality; PHIV: prevalence of HIV-positive; PDEF: percentual default of treatment; PR: prevalence of primary resistance + acquired resistance; MIG: migrations; OLDP: age of the population; AHS: adequate health services; DOTS: directly observed treatment short-course; EDU: educational level; NUT: nutrition level; HRTb: human resources for TB control; DPP: degree of political participation of the population.

Among all the components, social inequality should be emphasized as the most important one, since it generates poverty and, consequently, leads to malnutrition, ill-provided living conditions and education, among others, thereby influencing practically all other components. It should also be pointed out that the prevalence of primary resistance (whose definition is given below) stands as an aggravating epidemiological factor more important than acquired resistance (subsequently defined under treatment section). According to Ruffino-Netto (2004), the above-mentioned expression does not represent a mathematical equation and, therefore, will not yield predictable numeric solutions, but rather facilitates to wonder about the problem (TB charge analysis). One should notice that it contains variables of qualitative nature.

In nations such as India, China, Indonesia, Pakistan, Nigeria, Philippine, South Africa, Ethiopia, Vietnam, Russian Federation, Democratic Republic of Congo, Brazil, United Republic of Tanzania, Kenya, Thailand, Myanmar, Afghanistan, Uganda, Peru, Zimbabwe, and Cambodia, it is estimated an occurrence of up to 80% of world TB cases. It can be immediately perceived that, among the above-mentioned nations, the problem may be located either at the level of the numerator, denominator, or both within the expression. In Russia, for example, the multidrug-resistance prevalence problem is prominent and severe. In African nations, all the numerator and denominator components have a strong influence. Brazil might represent an intermediate condition, as it displays a (yet) small multidrug-resistance prevalence problem, but deals with major social inequality and has all remaining components in a phase of organization.

It is possible that a more elaborated reflection of this new "formula" might allow one to perceive that at the same time technical, biological, clinical, and epidemiological knowledge on TB advances, its social dimension should be remembered, valorized and used as an indicator of poor living conditions in other discussion forums such as The World Health Assembly, The International Labour Organization, and The World Trade Organization, among others.

Antibiotic treatment efficacy against Koch's bacillus

has had some hindrances, in part, owing to human natural reluctance in complying with the 6 month-intensive treatment required, and more recently, owing to development of drug-resistant strains of the bacillus (Young 1998). In 1969, the Medic Chief of the National Institute of Health declared in congress that "it was time to close the books about infectious diseases" (Bloom & Murray 1992). With a similar point of view, the TB problem was considered to be "resolved" by authorities, as the malady, physiopathology, diagnostic methods, therapeutic strategies and pharmaceutical resources were already well known (Ruffino-Netto 2002). Arrogant and low-priced behaviors like these have severe consequences, as millions of people suffer daily from the various diseases (infectious or not) that afflict humans.

M. tuberculosis

M. tuberculosis is the principal TB etiological agent in humans; it is a weak Gram-positive rod-shaped bacterium that has no flagellum, does not form spores nor produces toxins and has no capsule. The microbe's width and height vary from 0.3 to 0.6 and 1 to 4 μm, respectively, and it presents a complex cellular envelope, considerably slow growth, and genetic homogeneity. It is a macrophage intracellular pathogen that establishes its infection preferentially to the pulmonary system, where it is usually conditioned into a dormancy state as long as host's immune system prevails. The generation time is around 24 h in both synthetic medium and on infected animals (Cole et al. 1998). Bacterial growth at laboratory environment permits visual colony formation; these have a dry and wrinkled surface, and requires 3 to 4 weeks of growth on solid media to become visible (Bloom & Murray 1992). Owing to its pathogenicity and aerosol transmission, there are specific biosafety guidelines that demand the use of laminar-flow hoods and level 3 facility equipments for *M. tuberculosis* laboratorial work.

Currently, there are 60 known species among the *Mycobacterium* genera, most of them being saprophytic soil bacteria; and a minority of these species is pathogenic to humans, causing TB (*M. tuberculosis*, *M. bovis*, and *M. africanum*) and leprosy (*M. leprae*) (Jarlier & Nikaido 1994). The *M. tuberculosis* complex consists of *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. africanum*, and *M. microti*; and it is believed that their ancestral is a bacterium from soil, and that the human bacillus may have been derived from the bovine form (*M. bovis*), probably through cattle domestication by the pre-dynastic Egyptians. *M. tuberculosis* genomic studies indicate that horizontal transference of genetic material to a free living ancestral from the *M. tuberculosis* complex might have occurred in nature before the bacillus adopted its specialized intracellular niche (Cole et al. 1998, Daniel 1997).

There is a great variability in the infection rates among people exposed to different infection sources, and among infected ones, approximately 90% will never develop the disease at all. There are also differences in the capacity to transmit the infection among patients due to exhaled bacterial charge variability. Experimental evidence also suggests that the observed variation in transmission potential might be attributed to pathogenic features. *M. tuberculosis* can have virulence variations among different strains; the more virulent ones present higher pathogenicity or capacity to develop active disease. However, it should be pointed out that it is fundamental to have a clear distinction between the capacity a strain has to cause infection and its capacity to develop the disease (Bloom & Small 1998).

Like many other infectious diseases, TB presents epidemic cycles that might, although rarely, take centuries to end its course. History has proved that pathogenic virulence suffers considerable reduction along time, and natural selection takes place upon its hosts, as only the more genetically resistant ones survive. Since untreated TB patients have mortality rates ranging from 40 to 60%, one can consider the possibility of human selection occurrence in favor to the ones with increased genetic resistance (Bloom & Small 1998). Although genes will not influence the risk of infection upon exposure, they might determine the risk of disease development and its course (Daniel 1997). Furthermore, although genetic variability evidence on TB human susceptibility is very difficult to be determined, several studies revealed a greater disease severity among black than white people, there is a greater TB course agreement between monozygotic than heterozygotic twins, and populations more recently exposed to the TB bacillus have a greater propensity to develop the disease when compared to others that coexist with TB for centuries. As one gathers all these data, he can suppose that *M. tuberculosis* still is an extraordinary pathogen which exerts a powerful selective pressure on the human genome (Bloom & Small 1998).

M. tuberculosis genome

The complete genome sequencing of the best characterized *M. tuberculosis* strain, H37Rv, allowed the identification of unique microbial features. This pathogen has a circular chromosome with 4,411,529 base pairs with a 65.6% G+C content. Since its isolation in 1905, this strain has had a great application worldwide in biomedical research due to total TB virulence retention in animal models, and also because it is susceptible to drugs and amenable to genetic manipulation (Cole et al. 1998). *M. tuberculosis* sequence determination establishes a new phase in the battle against one of the more successful predators of the human race (Young 1998).

Although *M. tuberculosis* genome is smaller than *Escherichia coli*, it is very versatile, coding for most of the typical bacterial anabolic and catabolic pathways and amino acid synthesis/degradation. However, it is important to point out that a feature that differentiates *M. tuberculosis* from any other bacteria is the presence of a genome with approximately 4000 genes, mostly coding for enzymes involved in lipolysis (for bacterial survival inside its host) and lipogenesis (for cellular envelope synthesis). This microbe has around 250 enzymes involved in fatty acid metabolism. The genome is rich in repetitive DNA, especially insertion sequences, inserted in intergenic or non-coding regions, frequently close to tRNA genes. These are usually clustered, indicating the existence of insertion hot spots, which may prevent gene inactivation. There have been at least two prophages detected

in its genome, which can be involved with the fact that *M. tuberculosis* has a low lysis level in culture. Interestingly, about 59% of the genes are transcribed in the same direction as the replication; once compared to the 75% of *Bacillus subtilis*, one can probably relate this feature to the peculiar slow growth and infrequent replication cycles of the former one. Data comparative analyses allowed the precise function attribution to 40% of the proteins identified, and some information or similarities to another 44%. The remaining 16% have no similarity to any known protein, being probably involved in specific mycobacterial functions. Among secreted proteins that were identified in the mycobacterial genome sequence that could act as virulence factors are phospholipases C, lipases and esterases, which might attack cellular or vacuolar membranes, as well as several proteases. One of these phospholipases is involved in bacterial persistence in the nutrient-limited phagosome environment.

Stewart Cole et al. (1998) made an important contribution as they identified a group of variable elements, the polymorphic G+C-rich sequences, which correspond to a family of sequences that encode proteins with small peptide motifs, PE and PPE, which are organized as common repetitive domains. These proteins represent approximately 10% of the genome coding capacity and seem to be remnants of the ones related to antigenic variation in other bacteria. Through protein expression pattern alternation, these pathogens can be presented to their host's immune system as a moving target, interfering in the immunological response by antigen processing inhibition, and thereby, ensuring a greater survival probability to the bacteria.

M. tuberculosis proteome determination and its comparison with that of other microorganisms whose sequences are available revealed a significant statistical preference for the amino acids alanine, glycine, proline, arginine, and tryptophan, which are all encoded by G+C-rich codons, and a comparative reduction in the use of amino acids encoded by A+T-rich codons, such as asparagine, isoleucine, lysine, phenylalanine, and tyrosine. By means of mycobacterial genomic inspection, it became clear that besides the various functions involved in lipid metabolism, the enzymes required for glycolysis, the pentose phosphate pathway, and the tricarboxylic acid and glyoxylate cycles are also present, thereby evidencing the dynamic metabolism of the bacillus (Cole et al. 1998).

Mycobacterial cellular envelope

Mycobacteria produce an extremely uncommon cell wall structure; the peptidoglycan contains *N*-glycolylmuramic acid instead of the usual *N*-acetylmuramic acid, found amongst most other bacteria. A far more distinctive feature is that up to 60% of the mycobacterial cell wall is composed of lipids that consist basically of uncommonly long-chain fatty acids with 60 to 90 carbons, denominated mycolic acids (Brennan & Nikaido 1995). Mycolic acids are branched fatty acids that have a short and a long branch, with 22 to 24 and 40 to 64 carbons, respectively (Jarlier & Nikaido 1994); they are covalently linked to the polysaccharide that composes the cell wall, the arabinogalactan, which in turn is attached to peptidoglycan by a phosphodiester link (Brennan & Nikaido 1995). Approximately 10% of the arabinose residues in the arabinogalactan are substituted by mycolic acids (McNeil & Brennan 1991). The cell wall also contains several other free lipid species, which are not covalently attached to this basal skeleton (the mycolylarabinogalactan-peptidoglycan complex). These lipids can act as antigens in the host (Brennan & Nikaido 1995).

In 1982, Minnikin proposed a new cell wall model where the mycolic acid chains are packed side by side perpendicular to the cell surface, and this inner leaflet of long-chain fatty acids is covered by an outer leaflet composed of extractable lipids, thereby reproducing an asymmetric lipid bilayer. Recently, this model was updated by mycobacterial cell wall X-ray diffraction studies. In the ara-binogalactan polysaccharide, both galactan main chain and arabinan side branches are designed in a manner that would ensure maximum mobility between sugar residues. The mycolic acid residues are esterified to approximately two-thirds of the non-reducing termini of this highly branched polysaccharide (McNeil & Brennan 1991), as shown in Fig. 1.

Another distinguishing property shared among mycobacteria is the fact that their cell wall retain carbol fuchsin dye even in the presence of acidic alcohol, for this reason the rod-shaped mycobacteria are also known as acid fast bacilli (Glickman & Jacobs 2001). *M. tuberculosis* produces a considerably diverse array of lipophilic molecules, which range from simple fatty acids, such as palmitate and tuberculostearate, to long-chain complex molecules, such as mycolic acids and phenolphthiocerol alcohols (mycoside attachment) (Cole et al. 1998). Although mycobacteria have various cell wall lipid types, some are limited to specific species, such as sulfolipids, solely present in *M. tuberculosis* and which are involved in its pathogenicity (Brennan & Nikaido 1995). Furthermore, the mycobacterial cell wall fluidity gradient appears to have an opposite orientation to all Gram-negative bacteria, as the more external regions are more fluid than the internal ones (Brennan & Nikaido 1995).

Mycobacteria possess membrane proteins that form selective cationic channels called porins that control or retard the diffusion of small hydrophilic molecules, thereby conferring low cell wall permeability to hydrophilic solutes (Jarlier & Nikaido 1994). Amongst mycobacterial species, *M. tuberculosis* is one of the more permeable to hydrophilic antimycobacterial agents, and, thereby, less resistant to such drugs (for instance, ethambutol) (Brennan & Nikaido 1995). In principle, lipophilic molecules should be able to easily cross any biological membrane, dissolving itself in the hydrocarbon interior of the lipid bilayer. However, factors such as low fluidity of the mycolic acid leaflet and the bilayer's uncommon thickness result in reduction of this process in the mycobacterial cell wall. The inner leaflet presents a low fluidity, indeed a nearly crystalline structure; since the diffusion of lipophilic solutes through a lipid bilayer requires a fluid interior, this structure should act as an excellent barrier against the penetration of lipophilic antibiotics. Notwithstanding, the more lipophilic derivatives of chemotherapeutic agents are often more active against mycobacteria,

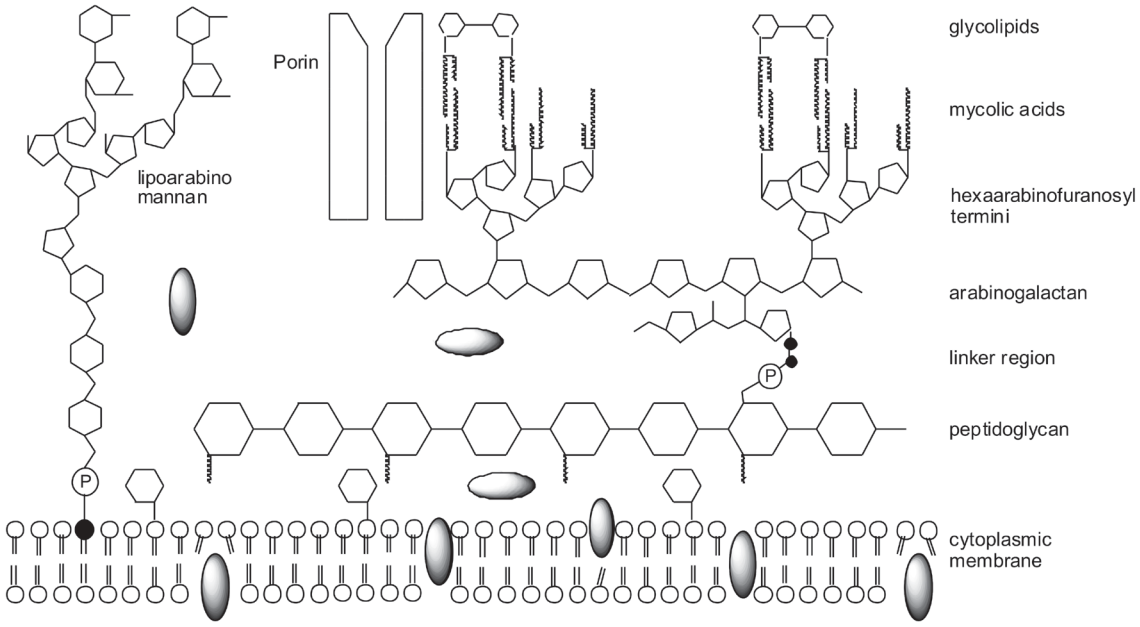

Fig. 1: schematic representation of the mycobacterial cell wall.

suggesting contribution of a lipophilic pathway to lipophilic solute transport (Jarlier & Nikaido 1994).

Since mycobacteria are relatively resistant to drying, alkali, and many other chemical disinfectants, it is thus very difficult to prevent *M. tuberculosis* transmission in urban institution environments. This resistance, and the resistance to therapeutic agents, are both basically conferred by the extremely uncommon mycobacterial cell wall structure (Brennan & Nikaido 1995). The unusual cell wall also permits the microorganism to survive inside the macrophage, which would usually destroy phagocytosed pathogens (NSB Editorial Comment 2000).

Although the cell wall acts as an exceptional permeable barrier, resistance to drugs in mycobacteria normally requires the participation of additional mechanisms, such as the removal of incorporated antibiotic molecules through chemical modification by β-lactamases that, synergistically, confer significant resistance levels (Jarlier & Nikaido 1994). Thereby, since *M. tuberculosis* has a cell wall with a relatively high permeability, the inactivation of the second factor in synergism may allow an effective chemotherapy. *M. tuberculosis* cell wall has become a target of the more recent researches towards the elucidation of the mechanism of action of many old drugs and the search of targets for the design of new ones (Brennan & Nikaido 1995). New data on genes specifically involved in its synthesis may represent potential drug targets (Young 1998).

Epidemiology and disease properties

Based on tuberculin skin test reactivity, epidemiologists estimate that around one third of the world population (1.7 billion people) is infected with *M. tuberculosis*, and at risk of developing active TB. Statistical data indicate the occurrence of 8 to 10 million new TB cases and 3 million deaths annually, afflicting mostly the young and productive adults. Under the current conditions, it is expected for this decade that 90 million people will develop the disease and 30 million will die from TB (Enarson & Murray 1996).

TB seems, to a certain point, under control in developed countries such as Japan and United States, but detains a violent manifestation in other places like southeastern Asia, Africa, and some regions of the Pacific, mostly due to complications of HIV infection and drug resistance (Brennan 1997). Approximately 95% of TB cases occur in developing nations, where 98% of the world TB death cases happen. According to the WHO databank, in 1998 Brazil occupied the 13th position among 22 countries where TB was well disseminated. In 1999, an interesting study of the distribution of TB notified cases among Brazilian states revealed a decreasing ordered incidence among São Paulo, Rio de Janeiro, Bahia, Minas Gerais, and Rio Grande do Sul (Ruffino-Netto 2002).

Currently, multidrug-resistant (MDR) TB presents a high incidence, in an increasing order, in Latvia, India, Estonia, Dominican Republic, and Argentina, and low incidence in most occidental European and African countries and United States (Fätkenheuer et al. 1999). The presence of resistant strains has a direct relation to drug availability and an inverse relation to treatment efficacy. A WHO and International Union Against Tuberculosis and Lung Disease (IUATLD) anti-TB drug-resistance global surveillance project made among 35 nations of 5 continents with standardized methods showed that during the 1994-1997 period, all the countries and regions analyzed presented *M. tuberculosis* strains resistant to at least one drug, usually isoniazid or streptomycin, suggesting that the disease represents a global problem (Pablos-Mendez et al. 1998).

Human TB is an infectious disease caused by some mycobacteria of the "*M. tuberculosis* complex", including *M. bovis*, *M. africanum,* and prevalently *M. tuberculosis*. According to the WHO, TB kills more people than malaria and AIDS together. Annually, TB is responsible for the death of 100,000 children worldwide, and 161,800 new cases occur only in Brazil. From now until 2020, it is estimated that 1 billion more people will be infected, 200 million will develop the disease, and 70 million will die in case surveillance and control strategies continue as they are (Pasqualoto & Ferreira 2001).

The principal means of transmission occurs by infective particles. Active TB patients will usually cough, as a result of typical chronic pulmonary inflammation, which constitutes the main dissemination mechanism for the pathogen to new hosts (Glickman & Jacobs 2001). The released particles from an ill patient are exhaled from the infected lungs into the air, being able to remain in suspension for hours, representing a highly contagious disease (NSB Editorial Comment 2000). Infection usually occurs from person to person through the inhalation of the infective particles (Pasqualoto & Ferreira 2001). Experiments with animal models demonstrate that particles in suspension containing 1 to 10 bacilli are enough to cause an infection. The main determinants of risk of infection are the concentration of bacilli in an exhaled particle from a source, its aerodynamic features, the ventilation rate, and the exposure period (Bloom & Murray 1992).

Usually, upon infection, inhaled bacilli are ingested by phagocytic alveolar macrophages, and can either be immediately eliminated or grow in the intracellular environment in localized lesions called tubercles. Two to six weeks past infection are usually followed by the establishment of cellular immunity, and subsequent lymphocyte and activated macrophage infiltration into the lesion, which leads to the elimination of most portion of the bacilli and the end of the primary infection, commonly without symptom presentation. The sole evidence of previous infection in these cases can be identified by the tuberculin skin test reactivity, or, in some cases, evidences of calcified lesions by X-ray.

In most cases, however, the bacilli can coexist pacifically within its human host as a quiescent or dormant form of infection, establishing a large bacterial reservoir among infected individuals. People harboring latent infection have an active TB developing risk of approximately 5% after the first year and 10% during their life-time.

Although much of the bacterial load is usually eliminated, a great proportion of the infiltrating phagocytes and lung parenchymal cells are also killed, which produces a characteristic solid caseous necrosis (granuloma or Gohn complex) where some bacilli have the opportunity to hide. In case host immune response predominates, the lesion is contained, causing simply residual damage to the lungs. However, in case the necrosis reaction expands, breaking into a bronchus, a lung cavity can be formed, which may allow a massive bacterial dissemination into the air through coughing. There can be even worse cases, such as when inflammatory cells liquefy the solid necrosis, creating a rich environment for bacillary proliferation (Bloom & Murray 1992, Young 1998).

Approximately 15% of the patients with the active disease present extra-pulmonary TB, which is caused by granuloma evolution due to excessive bacterial growth, invading the blood stream and disseminating the bacilli to various parts of the body. Also called miliary TB, it frequently occurs in the pleura, lymph nodes, liver, spleen, bones and joints, heart, brain, genital-urinary system, meningis, peritoneum, and skin.

The pathological and inflammatory processes produce typical TB symptoms such as weakness, fever, weight loss, night sweat, chest pain, respiratory insufficiency, and cough; advanced pathology may also cause blood vessel disruption, which leads to hemoptisis (Bloom & Murray 1992). For this reason, TB was also known as consumption, since the disease is developed at a leisurely pace and with multiple symptoms which lead to gradual debilitation and physical exhaustion.

Immune system in tuberculosis

Following intravenous mycobacterial infection in mice, the bacilli present (initially) a very short replication time in vivo, when macrophage activation begins by macrophage-derived pre-inflammatory cytokines, such as inter-leukin 6 (IL-6), IL-12, and tumor necrosis factor (TNF), besides the involvement of gamma interferon (INF-γ), initially derived from natural killer (NK) cells, in order to contain or inhibit bacterial growth. Approximately 2 weeks after initial infection, there is a considerable reduction of bacterial growth (representing a plateau in a growth plot) due to the activation and differentiation of specific lymphocytes, which are able to supply the lack of INF-γ required to increase the initial innate response, activating macrophages that induce the nitric oxide synthase 2 (iNOS) to produce nitric oxide, one of the main myco-bacteriostatic mediators or effector molecules in mice. Following infection with *M. tuberculosis*, there is a significant reduction of bacterial load in the liver, and also in the spleen (to a lesser extent). The remaining bacilli enter into a state of non-replicating persistence, although these still are fully viable (Ehlers 1999). Although the bacillus charge in this phase of the infection in mice does not mimic the latent state in the human host, it represents an equilibrium between the pathogen's persistence and the host's immune response (Glickman & Jacobs 2001). This dormant but yet viable bacillary form can re-establish its replication and develop the active disease in certain conditions of immune suppression, such as aging, corticosteroid therapy, CD4 cell charge reduction or treatments with iNOS inhibitors. Unfortunately, total bacillary elimination is improbable solely through the immune system, and considerably difficult to be reached by chemotherapy (Ehlers 1999).

Although most inhaled bacilli are usually rapidly destroyed by the host immune system, some will eventually establish infection, primarily in macrophages, inhabiting inside a membrane-bound vacuole, the phagosome (Glickman & Jacobs 2001). These can sometimes remain in a dormant form inside lung macrophages lodged in the calcified structures called tubercles (which gave origin to the disease's name), resultant of an attempt to isolate the

infected area (NSB Editorial Comment 2000). Granuloma formation might sometimes occur at the moment where the above-mentioned plateau is reached, causing destabilization and destruction of adjacent tissues, and possibly necrosis, followed by cavity formation (Jagirdar & Zagzag 1996). Granulomas are a result of CD4-mediated delayed-type hypersensitivity reaction within parenchymal tissues. Accordingly, the same system that is responsible for bacterial growth decrease (host defense) is also intrinsically associated with tissue damage through granuloma formation and necrosis (Ehlers 1999). Many TB symptoms, including tissue destruction which eventually liquefies lung infected portions, are preferentially mediated by the host's immune response against the bacillus instead of the bacterial virulence itself (Glickman & Jacobs 2001).

Depending on the bacterial charge that persists in the primary lesions, there can be granuloma development and differentiation. Owing to an efficient systemic antibacterial response, liver-localized granulomas will frequently suffer size reduction and can sometimes disappear. In the lungs, where bacterial charge is constantly high, it can be observed evident chronic-progressive pathology. Progressive interstitial fibrosis can eventually occur, gradually replacing most of the lung airspaces with dense fibrotic tissue separating groups of alveolar sacs, giving the entire lung a honeycomb appearance (Ehlers 1999).

M. tuberculosis latency and reactivation

Latent TB is a clinical syndrome caused by exposure to *M. tuberculosis*, followed by establishment of infection and host's immune response to control bacillary growth, forcing it into a quiescent state in the infected tissue. It is characterized by a reduction of bacterial metabolism, as a consequence of the action of cellular immune response, and which can, to a certain point, contain, but not eradicate, the infection. Contrary to active TB, latent TB is not an infectious disease and, therefore, does not represent a public health threat. Since latent TB is not presented as a clinical illness, the sole form to be diagnosed is by tuberculin skin tests. A positive result indicates that the patient has already had contact with the microbe. The method relies on the intradermal inoculation of PPD. In certain cases, the infection can be identified through a chest X-ray radiography that demonstrates scars of an old infection.

The bacterial intracellular survival is based on its capacity to deal with the phagosome acidification in infected macrophages and prevent the phagosome-lysosome fusion. In most immunocompetent infected patients, there is the occurrence of T-cell and macrophage recruitment, and the establishment of secondary immune response, resulting in infection control. As the immune system begins to fail, latent infection can be reactivated, leading to the development of active TB, frequently several decades after initial infection. Reactivation can be induced by various factors, all of which compromise the immune system's efficacy, such as HIV co-infection, malnutrition, aging, drug use, cancer, diabetes, chronic renal insufficiency and immunosuppressive drug therapy (Parrish et al. 1998).

It is well known that the conventional treatment can reduce active TB risk in patients recently infected by the bacillus. Since 90% of the infected people develop immune response against the microorganism, it becomes interesting to establish an effective protection mechanism that complements the immune system action, curbing disease development through drugs or recombinant strain vaccines. Antigenic expression induction during latent infection can optimize immune system activation against microorganisms that insist in persisting (Young 2001).

Tuberculosis and AIDS

The connection between TB and the human HIV was well documented for the first time in New York, where it was estimated that the risk of developing active TB among HIV and *M. tuberculosis* co-infected people was approximately 8% per year, compared to 10% risk throughout life-time for people infected solely with the bacillus (Bloom & Murray 1992). As the immune system withers due to HIV infection, the probability to develop the disease increases up to 30 times (Pasqualoto & Ferreira 2001). An increase in TB susceptibility is associated to the first HIV infection stages, accelerating its progression to acquired immune deficiency syndrome (AIDS) (Young 1998). The incidence of double infection cases occurs mainly among workers in high productivity age, such as 15 to 59 years old (Narain et al. 1992). Among patients with AIDS (immune suppressed) there can be opportunistic infections, caused by the so called "atypical mycobacteria", which include the *M. avium* complex, *M. kansasii*, *M. fortuitum*, and *M. chelonae*, although these species are essentially saprophytic (Brennan & Nikaido 1995).

Currently, HIV infection represents the major risk for the progression of a latent infection into the active disease. Furthermore, TB induces AIDS development in HIV-positive patients by the production of stimulatory cytokines and a decrease in CD4 cell charge. Therefore, HIV-*M. tuberculosis* co-infection represents a devastating problem to both, infected patients and the global population. In the last decades, a dramatic increase in TB incidence in Africa has been observed, as a consequence mainly of HIV epidemic, reaching alarming rates and leading people to question whether "Africa can still be saved". A study performed in this continent demonstrated that there are several evidences that demonstrate the strong association between these two infections, as: TB is the most important disease associated to HIV and AIDS-defining disease; TB is the principal cause of mortality among patients HIV-infected in hospitals; TB incidence increase coincides within period, region and population with the appearance of AIDS; HIV is more present among people with TB than within the whole population; active TB is more common between HIV-positive individuals than negative ones. There are approximately 20 million people infected with HIV in sub-Saharan Africa, and half of these are co-infected with TB. According to the WHO, one third of the increase in TB incidence in the last 6 years was attributed to HIV co-infection. In Brazil, it is estimated that 200,000 people are co-infected with TB and HIV. The synergism established between these two infections rendered them the designation of "cursed duet".

HIV infection has drastically changed TB's epidemiology and "natural" history, causing an increase in its transmission dynamic, morbidity and mortality. TB diagnosis among HIV-positive patients became more difficult to be performed due to various factors, such as: false negative tuberculin skin tests; pulmonary TB with atypical chest X-ray findings or with sputum smears negative for acid fast bacilli; and extra-pulmonary TB (Fätkenheuer et al. 1999). It is evident that HIV epidemic favors the emergence of drug-resistant strains of the TB bacillus in co-infected patients since, in these cases, there is a higher treatment abandon rate (Brennan 1997). Mortality rates among HIV-positive patients infected with MDR-TB frequently exceed 80%, and the period between the diagnosis and death usually ranges from 4 to 16 weeks (Riley 1993). For these reasons, MDR-TB is currently known as "the most malignant opportunistic infection yet associated with HIV infection" (Nolan 1997).

Diagnosis

The tuberculin skin test is the epidemiological surveillance method currently disseminated throughout the world, and can be used to detect infections from many years past or even very recent ones (Bloom & Murray 1992), and it is the only way to detect a latent infection by the Koch's bacillus, through delayed type hypersensitivity against mycobacterial antigens (Glickman & Jacobs 2001). However, BCG vaccination also produces reactivity to PPD, making the use and trustworthiness of this method gradually lower as child BCG vaccination increases (Bloom & Murray 1992). It was demonstrated by an animal model experiment that there is a great discrepancy between immunological, bacteriological and microscopic methods for the detection of latent TB in infected animals, which suggests that none of these techniques is 100% sensitive. Based on this fact, it can be inferred that the tuberculin skin test used to detect latent TB in humans gives distrustful results (Ehlers 1999).

Diagnosis and treatment of pediatric TB have several difficulties due to various factors, since young children rarely expectorate, tuberculin skin tests are not always easily interpreted, as false positive results can occur due to BCG vaccination, which is routinely given at birth; and false negative results can frequently occur in immune suppressed patients (Fätkenheuer et al. 1999). In order to diagnose MDR-TB, it is necessary to perform bacillary sensibility tests for anti-TB drugs (Bactec system), using established drug concentrations, and a control without drugs (as a reference). Another technique is the proportion method, in which it is defined which drugs and at what minimal concentrations occurs inhibition of at least 99% of bacterial growth (Petrini & Hoffner 1999). The progress of molecular techniques allowed the development of more sensitive and rapid methods for the detection and identification of mycobacteria. Many of these methods are commercially available, with sensitivity and specificity usually superior to 90%. However, one of the main problems here relies on the cost of these methods, restricting their use to developed nations. Molecular detection methods usually initiate with genomic sequence amplification, generally through polymerase chain reaction (PCR), which uses specific repetitive or DNA single copy sequences, and can give a specific and sensitive diagnosis in a few hours (Bloom & Murray 1992, Caws & Drobniewski 2001). In vitro amplification of specific sequences in the pathogen's genome allows a rapid diagnosis with a greater sensitivity and specificity degree than standard traditional methods that have been established along the past years. In a few hours, it is possible to identify relevant pathogenic clinical features, either directly in samples or in precocious cultures, and detect antimicrobial resistance markers directly on samples. Thereby, TB diagnosis can now be confirmed in a single day instead of a 1 to 2 month period as in the past.

There are many mycobacterial genes that confer resistance to drugs caused by specific mutations. After the sequencing of these genes and the identification of their mutations, one can use several molecular detection methods for drug resistance. Although the ideal one would be DNA sequencing, it is sometimes impracticable, forcing the option for alternative techniques, such as PCR single-strand conformation polymorphism analysis, heteroduplex analysis, mutation-specific priming, restriction enzyme analysis, and solid-phase hybridization methods. Other rapid detection systems have recently been developed as an alternative based on phenotypic methods, which can be adapted for use in susceptibility tests. One of these techniques uses mycobacteriophages with the *lux* gene inserted in the genome, for example; other methods include flow cytometry and reverse transcriptase PCR (Caws & Drobniewski 2001).

Molecular methods tend to have their use gradually increased in order to perform rapid diagnosis, pathogenical studies and epidemiological distribution of infectious diseases. The availability of genomic sequences from a great number of microbial pathogens will provide a better understanding of their evolutive genetics, virulence and interactions with its host (Gilbert 2002). Although molecular diagnosis has a modest prestige, human disease diagnosis is clearly tending towards these innovative methods. According to Daniel Farkas, this molecular trend is due to the genomic Era, which turned available genomic sequences from important organisms, and also to the discovery of relevant targets to diagnose (Farkas 2002).

In order to have a better understanding of pathogenicity, it becomes necessary to know the classes or groups of proteins and their variants. Therefore, proteomic use and development becomes necessary in order to explain complex disease phenotypes. The identification of all protein variants, their inter-relations, and the functional consequences generated by changes in their levels should be considered to understand the clinical presentation of complex diseases. There are some barriers to be overcome before there can be an implementation of molecular diagnostic tests in clinical laboratories, such as: which test to be applied, the technology and equipment to be chosen, and factors like cost-effectiveness, precision, and staff training, among others (Fortina et al. 2002).

It is already known that drug efficacy or toxicity varies among medicated patients and, since these differences are greater among people with no sort of kinship than

between monozygotic twins, it is believed that some of these differences in drug response are inherited. It is supposed that this can be attributed to polymorphisms in genes encoding drug-metabolizing enzymes, drug transporters and/or drug targets. However, it is recognized that many non-genetic factors influence the effects of medications in patients, including the nature and the severity of the disease that is being treated, the patient's age, sex, and ethnicity, among others. The utility of pharmacogenetics in diagnosis will come from the ability to determine, based on genetic tests, the probability of a specific medication to produce the desired therapeutic effects (the efficacy) or the risk of an adverse response to the drug (the toxicity). It can divide a population of patients with the same diagnostic into sub-groups that have genetic differences in their metabolism and/or drug response susceptibility.

Chemotherapy efficacy rates to most diseases vary from 25 to 80%. Therefore, the ability to predict an efficient response based on genetic tests, performed before the beginning of therapy, has the potential to be of great clinical value. Since all drugs that produce an efficient response can, in specific conditions, induce adverse effects, the availability of genetic tests that can identify the patients at risk of developing the rare, but still severe, adverse effect seems particularly attractive. These genetic determinants of drug effects remain stable during the patient's lifetime, and, therefore, require a single measurement. At birth the child can have a blood sample collected in order to have the genome determined, which can be used throughout life to guide primary prevention strategies, make diagnoses on a molecular basis, and establish particular drug therapy, that is, translate functional genomics into personalized medicine. It seems inevitable that, in the future, pharmacogenomics will come up to be an important part in the process of drug development (Johnson & Evans 2002).

Treatment

It happens then as it does to physicians in the treatment of Consumption, which in the commencement is easy to cure and difficult to understand; but when it has neither been discovered in due time nor treated upon a proper principle, it becomes easy to understand and difficult to cure. The same thing happens in state affairs; by foreseeing them at a distance, which is only done by men of talents, the evils which might arise from them are soon cured; but when, from want of foresight, they are suffered to increase to such a height that they are perceptible to everyone, there is no longer any remedy.

Nicollo Machiavelli, in The Prince, Luigi Ricci, Ed. (English edition, Oxford University Press, Oxford, 1933), chap. 3, p. 153.

About half of the new TB cases would be inevitable, as a consequence of the disease's natural history and HIV co-infection. However, many of the exceeding cases, which result from the increase in active transmission, could be prevented by effective treatment program implementation (Bloom & Murray 1992). In the XIX century, common "treatments" for TB included lung collapse and thoracoplasty (mutilating surgery where a few ribs were removed from the patient with cavitary TB), besides the isolation of infected patients to institutions, where the air was thought to be cleaner (NSB Editorial Comment 2000). Previously, in the classic period of the Roman Empire, Clarissimus Galenus, son of the great mathematician and architect Nikon, used to prescribe to his patients treatments based on fresh milk, pure air, maritime trips, horseback rides, and much rest in dry environments of higher altitude. TB treatment has evolved throughout time, from magical potions to rational drug use (Daniel 1997).

The principal objective of chemotherapy in TB patients is the eradication of the whole bacillary load (Petrini & Hoffner 1999). The disease is caused by a well studied pathogen, against which there can be "magic bullets" – drugs that eliminate the bacteria without causing damage to man (Daniel 1997). Modern therapy relies on a combination of potent bactericidal agents, such as isoniazid, rifampicin and pyrazinamide, in a treatment with six month duration. Sometimes during treatment, there can be an initial resistance of the bacillus to isoniazid, making it necessary to add other first-line drugs to the treatment, such as ethambutol and streptomycin. Whenever there is resistance to at least rifampicin and isoniazid, which characterizes MDR-TB (Telenti & Iseman 2000) it becomes necessary to extend the treatment period, and frequently rely on the use of second- or even third-line drugs, even though the increased toxicity stands as a negative factor. Very recently the Center for Diseases Control, CDC-USA, defined a new class of MDR, which was named extensively drug-resistant (XDR) TB, whose isolates were resistant to isoniazid and rifampicin and at least three of the six main classes of second line drugs (aminoglycosides, polypetides, fluoroquinolones, thioamides, cycloserine and para-aminosalicylic acid) (CDC 2006). In extreme cases, when an infection involves MDR or XDR strains (or when there is a portion of an organ considerably damaged), it becomes sometimes necessary to resort to surgical removal of granulomas, as an attempt to increase the likelihood of cure (Telenti & Iseman 2000).

First-line drugs are mainly bactericidal, and combine a high degree of efficacy with a relative toxicity to the patient during treatment; these include isoniazid, rifampicin, streptomycin, ethambutol, pyrazinamide, and fluoroquinolones (Fig. 2). Second-line drugs are mainly bacteriostatic, which have a lower efficacy and are usually more toxic; these include para-aminosalicylic acid, ethionamide, and cycloserine (Fig. 3), among others (Goodman et al. 1996). Effective TB chemotherapy must include early bactericidal action against rapidly growing organisms and subsequent sterilization of dormant populations of bacilli. The first-line drugs exhibit early bactericidal activity against actively metabolizing bacilli and the bacteriostatic second-line drugs are reserved to strengthen the treatments with the presence of resistance (NSB Editorial Comment 2000). Among all first-line anti-TB agents, isoniazid has the greatest bactericidal activity against microorganisms growing actively in cavities, followed by rifampicin, streptomycin and quinolones. However, isoniazid can frequently cause fever, and is one of the drugs with the highest degree of toxicity (Morehead 2000).

Although TB is a serious illness, it can still be cured in most new cases as long as, once diagnosed, appropriate

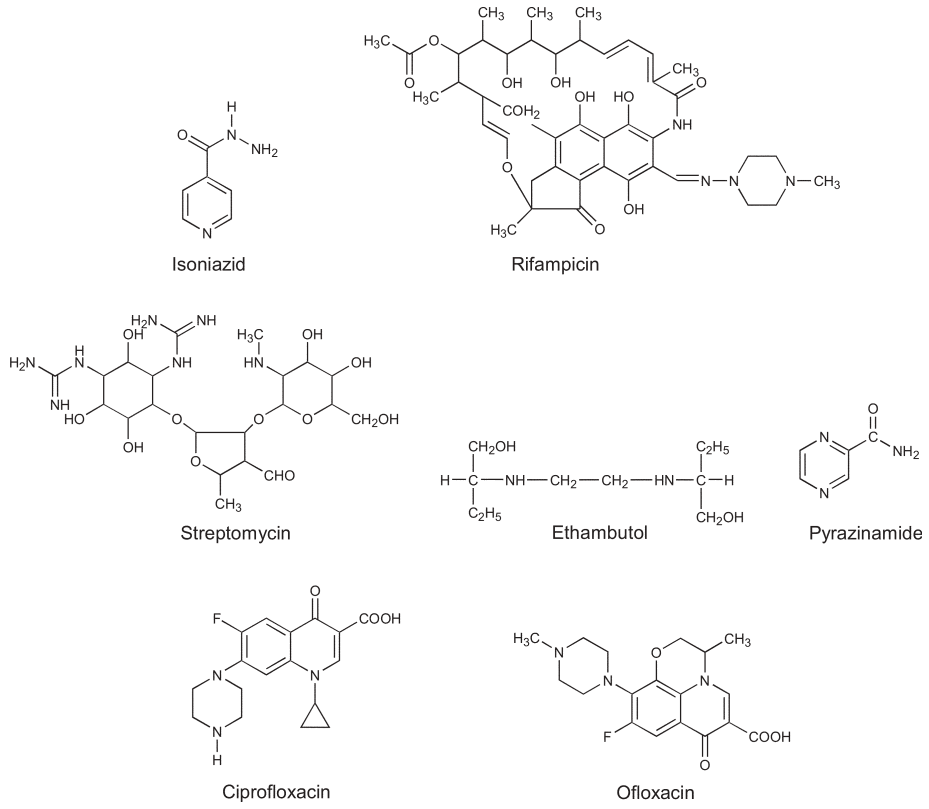

Fig. 2: first-line drugs are mainly bactericidal, combining a high efficacy with a relative low toxicity to patients undergoing treatment.

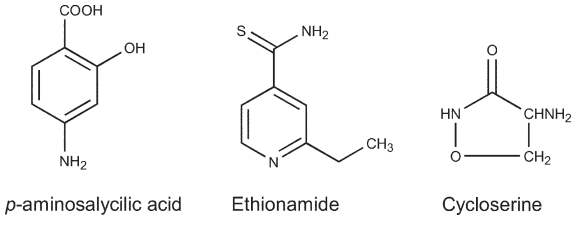

Fig. 3: second-line drugs are usually more toxic than first-line ones, have a lower efficacy and are mainly bacteriostatic.

chemotherapy is employed, since one of the greatest risks of mortality in TB is the delayed treatment. The treatment is normally consists of a medicinal association of regular use for a period long enough to avoid bacterial resistance and persistence. The treatment currently recommended by the WHO consists of the combined administration of isoniazid, rifampicin, pyrazinamide and streptomycin (or ethambutol) during the first 2 months, followed by the combination of isoniazid and rifampicin for at least 4 additional months. However, the long treatment period involves undesirable side effects to the administered drugs, leading patients to "give up" chemotherapy. The patient non-compliance led the WHO to invest in universal treatment adherence programs, through a process currently known as the directly observed treatment short-course (DOTS), where health care workers counsel patients, perform progress surveillance, and make sure that each medication dose is correctly taken (NSB Editorial Comment 2000).

This revolutionary treatment aims initially at a bacteriostatic action, inhibiting the synthesis of cell wall, nucleic acids and mycobacterial proteins, and, thereby, leading to a rapid elimination of most part of the infecting bacilli. The therapy also aims at a subsequent bactericidal action to consolidate the treatment through the elimination of all remaining bacilli. DOTS combines five fundamental elements: political commitment, microscopic services, drug supplies, surveillance systems and direct treatment observation. This strategy prevents the occurrence of new infections and, more importantly, makes MDR/XDR-TB generation impractible (Pasqualoto & Ferreira 2001).

Patients with latent TB can also be treated on a chemotherapic basis; this treatment is based solely on isoniazid (monotherapy) and works only for patients with the latent infection that have not developed the active disease yet. According to the pediatrician Edith Lincon, inventor of this treatment in 1954, the therapy relies on the prophylactic administration of isoniazid for 6 to 9 months; this treatment makes disease development unlikely due to dormant bacilli elimination. The patients that most benefit from this kind of therapy are the ones more recently infected and not at advanced age. Although this chemotherapy has reached excellent results in the United States, where it is currently widely used, this type of prevention was not adopted by practically any other nation

as part of the TB national control program (Daniel 1997).

HIV-positive patients treated according to the WHO recommendations (DOTS) have sputum conversion and cure rates similar to HIV-negative treated patients. According to the WHO, there are no treatments currently available that have yielded definitive cure results for active TB patients and MDR/XDR-TB control better than DOTS (Fätkenheuer et al. 1999). Even though the efficacy of the treatment for HIV-positive and negative patients is quite similar when dealing with drug-susceptible *M. tuberculosis* infection, the choice for an appropriate TB treatment when drug resistance is suspicious can frequently be affected by the lack of rapid diagnosis tests. Drug-susceptibility tests require approximately 8 weeks to be concluded, a period usually greater than co-infected patient's survival mean time (Riley 1993). Primary or initial resistance is defined by the identification of tolerance in individuals without previous medication; and acquired or secondary resistance is defined as the resistance resultant from previous inefficient treatments (Telenti & Iseman 2000). Usually, nations with a high primary resistance rates indicate the inefficiency of previous national TB control programs to curb the occurrence of transmission of resistant strains; secondary resistance to at least one drug is likely to reflect problems in programs in progress (Petrini & Hoffner 1999).

Curiously, the recognition of TB as a global threat and the interest in tackling this problem do not derive primarily from public health institutions, but rather from the World Bank and, most of all, by the IUATLD, which annually invests 4 million dollars in the establishment of control programs that detect approximately two-thirds of all cases, treat 65,000 cases, and provide cure rates ranging from 80 to 85% in some developing nations (Bloom & Murray 1992). However, this investment is still considerably below what is considered as necessary.

Since TB mortality is mainly attributed to the delayed detection of the disease, and is also associated to drug resistance and HIV co-infection, among others, it is expected that patients without these factors will survive. However, the treatment of patients infected with drug-susceptible *M. tuberculosis* that have developed active disease with sub-therapeutic drug doses can easily lead to death, as the bacilli have a favorable condition to expand infection and cause active disease (Morehead 2000).

Mechanism of drug action and MDR/XDR

M. tuberculosis is naturally resistant to many antibiotics and chemotherapeutic agents, such as β-lactams, owing to the presence of hydrolytic and drug-modifying enzymes, such as periplasmic β-lactamases and aminoglycoside acetyltransferases, and drug efflux systems, besides the fact that they possess a highly hydrophobic cell wall that acts as a contention barrier that makes treatment more difficult. This bacterium is only susceptible to isoniazid, ethambutol, aminoglycosides (such as streptomycin) and rifamicins (such as rifampicin), among antibiotics, and to fluoroquinolones (Fig. 2) among general chemotherapeutic agents (Brennan & Nikaido 1995). Within the same genus, obligatory parasite species such as *M. tuberculosis* have a relatively high permeability as compared to soil mycobacterial species that have developed a protection mechanism through the production of a cell wall with extremely low permeability, and, therefore, naturally more resistant (Jarlier & Nikaido 1994).

Many pathogenic bacteria possess resistance plasmids, which can effect a rapid MDR transition to drug-susceptible wild-type strains, and might confer resistance to many antibacterial substances at once. This has never been observed in *M. tuberculosis*, but it is known that the resistant and multi-resistant phenotypes are caused by random chromosomal mutations in different genes of this organism, such as nucleotidic insertions, deletions, or substitutions (Petrini & Hoffner 1999).

The drug resistance in TB treatment is almost as old as the introduction of the first anti-TB drugs (Petrini & Hoffner 1999). After 4 to 6 weeks of treatment, the physical debilitation symptoms begin to disappear, inducing many of the patients to interrupt the therapy. However, many of them end up developing the disease recurrently, making it necessary to initiate a new treatment, in case they are diagnosed, which creates favorable conditions for the selection of drug-resistant organisms (Bloom & Murray 1992). The first step towards the development of molecular detection methods of drug-resistance was the identification of genes and mutations involved in this process. Mycobacteria develop resistance to drugs spontaneously ("natural resistance"), and present different mutation rates for each drug. In the TB bacillus, these rates are equivalent to 1 in 10^5 to 10^6 for isoniazid, 1 in 10^8 for rifampicin (Riley 1993), 1 in 10^8 to 10^9 for streptomycin, 1 in 10^7 for ethambutol, and 1 in 10^9 for cycloserine (Gangadharam 1984). A cavitary lung lesion can shelter up to 10^9 organisms, and, thereby, it is probable that there exists isoniazid or rifampicin resistant organisms. Mutation rates for both drugs is 1 in 10^{14}, so it is virtually impossible for *M. tuberculosis* to become spontaneously resistant to both drugs in patients correctly treated (Riley 1993). As monotherapy induces the selection of drug-resistant populations ("acquired resistance"), it becomes necessary to use a combined therapy, since the probability of a bacterial strain to develop resistance to two or more drugs at the same time is extremely low (Petrini & Hoffner 1999).

The first biochemical effect of isoniazid occurs in the first stages of mycolic acid synthesis. Isoniazid is a synthetic pro-drug that requires the product of the *katG* structural gene for its activation (Telenti & Iseman 2000); this drug becomes an active compound once it is metabolized by the *M. tuberculosis* catalase-peroxidase enzyme, and inhibits the activity of the enoyl-ACP (CoA) reductase enzyme (encoded by the *inhA* gene) in the presence of NADH or NAD^+ (reviewed in Basso & Blanchard 1998, Schroeder et al. 2002, Basso & Santos 2005). Resistance to isoniazid is more complex, as it involves at least 4 genes: *katG*, which mediates both susceptibility and resistance to isoniazid, and encodes the catalase-peroxidase enzyme; *inhA*, which is involved in the elongation of fatty acids (Zhang et al. 1992); *ahpC*, which encodes the hydroperoxide alquil reductase C; and *oxyR*, which is an important regulator of oxidative stress (Telenti & Iseman 2000).

Rifampicin interacts specifically with the β subunit

of the RNA polymerase enzyme from prokaryotic organisms to inhibit transcription, leading to bacterial death. Molecular detection of resistance is relatively easy to be analyzed, since about 96% of the rifampicin-resistance cases involve specific mutation in the *rpoB* gene, which codes for the enzyme's beta chain, producing resistance to the drug through the decrease in binding affinity of rifampicin to the polymerase (Telenti et al. 1993). The mutation rate responsible for isoniazid resistance is 100 times greater than the one responsible for rifampicin resistance, and is usually the first modification in the susceptibility of wild-type *M. tuberculosis* (Petrini & Hoffner 1999). Since, in the United Kingdom, 90% of the rifampicin-resistant isolates are also isoniazid-resistant, a single positive resistance result to the former can be considered a strong indicator of MDR-TB (Caws & Drobniewski 2001).

Streptomycin acts as an inhibitor of prokaryotic protein synthesis initiation. Two genes were identified as being involved with the resistance to this drug: *rrs*, which encodes the rRNA 16S; and *rpsL*, which encodes the ribosomal protein S12 (Telenti & Iseman 2000). Resistance to ethambutol is determined by mutations in the *embA*, *embB* and *embC* genes, which encode enzymes involved in the synthesis of arabinan (Alcaide et al. 1997). Pyrazinamide is a drug that functions only against *M. tuberculosis*, and no other mycobacterial species. Pyrazinamide resistance appears to be conferred by mutations in the *pncA* gene, which encodes pyrazinamidase, an enzyme that hydrolyses the drug to turn it active (Telenti & Iseman 2000). Current experimental evidence indicates that pyrazinamide enters *M. tuberculosis* by passive difusion, is converted to pyrazinoic acid by pyrazinamidase/nicotinamidase enzyme activity, and is excreted by a weak efflux pump (Zhang & Mitchison 2003). Protonated pyrazinoic acid is then reabsorbed into the bacilli under acidic conditions and accumulates due to the inefficiency of the efflux pump, leading to cellular damage. Pyrazinoic acid and pyrazinamide could de-energize the membrane by collapsing the membrane potential and affect the membrane transport function at acidic pH. Unlike other antitubercular agents, PZA has no defined target of action. The mode of action of fluoroquinolones is on enzymes responsible for DNA topological conformation, topoisomerases, mainly DNA girases. Resistance to ciprofloxacine, one of the most active fluoroquinolones against *M. tuberculosis*, is conferred by mutations to *gyrA, gyrB* and *lfrA* genes, which encode the subunits A and B of DNA girase, and an efflux protein, respectively. Like most wild-type *M. tuberculosis* strains, many of the multiresistant strains are susceptible to fluoroquinolones, since these bactericidal compounds increase the activity of isoniazid and rifampicin (Telenti & Iseman 2000). Some second line drugs, such as cycloserine, should be used in the lack of alternatives, as these present greater toxicity and, thereby, can cause dangerous psychic collateral effects (Petrin & Hoffner 1999).

The rate of MDR-TB mortality is estimated to range from 40 to 60%, which is similar to the mortality of patients with untreated TB (Bloom & Murray 1992). Among some of the factors responsible for the increase in MDR/XDR-TB incidence, there is the HIV/AIDS epidemic and the increase in TB incidence, especially in populations with easy access to anti-TB medication. The principal risk factors that contribute for MDR/XDR-TB emergence are patient noncompliance with the treatment and the inappropriate administration of drugs by clinicians (Riley 1993). The Era of antibiotics has been constantly marked by cycles, which consist on the introduction of new antimicrobial agents and the subsequent emergence of resistance to these drugs (Swartz 2000).

Although MDR/XDR-TB patients manifest the disease in a more aggressive form, there is no evidence that these patients, HIV co-infected or not, are more prone to transmit the infection than patients with drug-susceptible TB, nor that these drug-resistant strains are more infectious than drug-susceptible ones. In HIV-negative patients, MDR/XDR-TB frequently leads to considerable loss of weight, respiratory insufficiency, and the formation of lung cavitary lesions. In contrast, in HIV-positive patients, MDR/XDR-TB is more aggressively developed, leading to high mortality rates (Riley 1993). A study performed in Florida with HIV-positive patients revealed that, since diagnosis, the mean duration of survival is approximately 45 days for MDR-TB co-infected patients and 430 days for drug-susceptible TB co-infected ones (Fischl et al. 1992).

The WHO estimates that around 50 million people are infected with MDR-TB, which is more difficult and expensive to be treated, and more likely to be fatal. In industrialized nations, the complete treatment costs about US$ 2,000 per patient, much cheaper than the US$ 250,000 required to treat an MDR-TB patient (Pasqualoto & Ferreira 2001). *M. tuberculosis* resistant strains are usually scarce in regions where there is little availability of drugs to fight the disease, since non-treated TB patients either die, get spontaneously cured, or become chronic bacilli disseminators, but, in any case, their infecting bacteria will rarely develop any kind of drug-resistance. In contrast, easy drug access and inappropriate chemotherapy conditions will always inevitably lead to drug-resistance (Petrini & Hofnner 1999). Therefore, MDR/XDR-TB is less common in developing nations, although high TB rates are usually present, owing to the lack of medications. However, among developed nations, where there is a greater availability of anti-TB medications, the MDR/XDR-TB rates tend to be much higher (Riley 1993). Inappropriate chemotherapy is defined as the use of a single drug, inappropriate combinations of drugs, short treatment periods resultant of patient noncompliance, and low absorption of the administered drugs. In these conditions, *M. tuberculosis* will be exposed to sub-lethal antibacterial concentrations, which impose a selection favorable to the growth of resistant bacilli among an originally susceptible population. When dealing with the detection or suspicion of resistance, one should avoid the addition of a single drug into the treatment, even though it shows an initial activity, as this situation resembles a monotherapy, which allows the development of resistance to a drug to a bacterial strain already resistant to a different agent (Petrini & Hoffner 1999).

In principle, *M. tuberculosis* genomic sequence includes information about all the possible targets to which

new antimycobacterial agents might be directed. Structural and functional information about a particular protein target can be, to a certain point, deduced by the sequence of the respective encoding gene. A way to exploit these information for drug development programs is by cloning and expressing genes that encode specific biosynthetic enzymes. The recombinant proteins can be used in functional assays for inhibitor search, and, at the same time, allow the generation of structural information for the development of drugs (Young 2001).

The *inhA*-encoded 2-*trans* enoyl-acyl carrier protein reductase enzyme (InhA) has been shown through biochemical and genetic studies to be the primary target for isoniazid. In agreement with these results, mutations in the *inhA* structural gene have been found in isoniazid-resistant clinical isolates of *M. tuberculosis*. In addition, the isoniazid-resistant InhA mutants were shown to have higher dissociation constant values for NADH and lower values for the apparent first-order rate constant for isoniazid inactivation as compared to wild-type InhA (Basso et al. 1998). More recently, an understanding of isoniazid drug resistance mechanism in *M. tuberculosis* was revealed by our group (Oliveira et al. 2006) through crystallographic and pre-steady-state kinetics studies on binding of NADH to wild-type and isoniazid-resistant enoyl-ACP(CoA) reductase enzymes from *M. tuberculosis*. In this work, in an attempt to identify structural changes between wild-type and isoniazid-resistant InhA enzymes, we have solved the crystal structures of tetrameric wild-type, S94A, I47T and I21V InhA proteins in complex with NADH to resolutions of, respectively, 2.3 Å, 2.2 Å, 2.0 Å, and 1.9 Å (Fig. 4). These InhA mutants were identified in isoniazid-resistant clinical isolates of M. tuberculosis (Basso et al. 1998). The more prominent structural differences are located in, and appear to indirectly affect, the dinucleotide binding loop structure. Moreover, studies on pre-steady-state kinetics of NADH binding have been carried out. The results showed that the limiting rate constant values for NADH dissociation from the InhA-NADH binary complexes (k_{off}) were 11-, 5-, and 10-fold higher for, respectively, I21V, I47T, and S94A isoniazid-resistant mutants of InhA as compared to isoniazid-sensitive wild-type InhA. Accordingly, these results are proposed to be able to account for the reduction in affinity for NADH for the isoniazid-resistant InhA enzymes. These results are in agreement with our molecular dynamic simulation studies that show a higher flexibility of pyrophosphate moiety of NADH and a lower occupancy of conserved direct bonds between the coenzyme and protein residues in isoniazid-resistant mutant than in wild-type InhA (Schroeder et al. 2005).

As one analyzes the pharmacodynamic parameters for anti-TB drugs, it becomes clear that isoniazid and rifampicin are considerably more potent than the other available drugs used. Clinical data proved that MDR/XDR-TB requires a period considerably longer to cure. Ethionamide can be considered the least potent drug among all, and considering the fact that its use tends to cause gastrointestinal irritation, this second-line drug is usually used as a last option. There are other factors that can affect the selection of anti-TB drugs, such as pregnancy and lactation, which are situations where the toxicity should be considered. Ethionamide is known to be teratogenic, and the aminoglycosides have been associated with decreased hearing in newborns. Accordingly, these agents should be avoided during pregnancy unless the survival of the mother depends of its use.

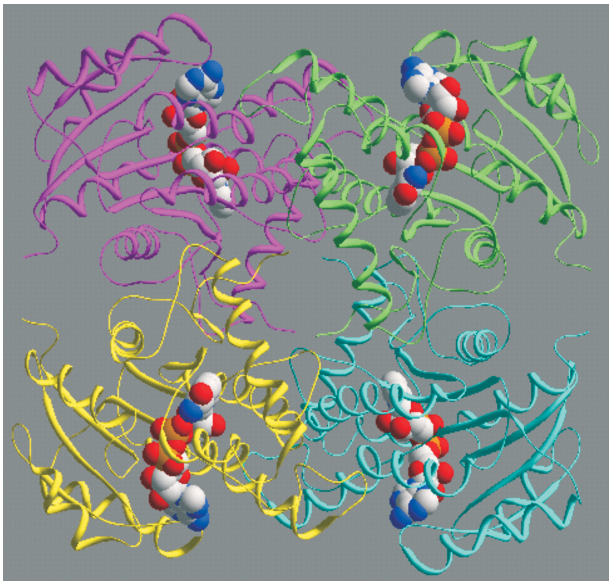

Fig. 4: three-dimensional structure of tetrameric wild-type enoyl-ACP reductase from *Mycobacterium tuberculosis* in complex with NADH (CPK model) refined to 1.92 Å (PDB access number 2AQ8, released on May 23rd, 2006).

For a drug treatment, there is no "right" dose for all patients. Usually, dosage of a particular drug is based on clinical considerations as a function of the patient's needs for the drug. The initial dosage regimen can then be tested in the patient, and the serum concentration can be measured. Subsequently, the doses can be adjusted as a function of the patient's needs. The pharmacodynamic relationship between the serum drug concentration and the probability of therapeutic response is described mathematically by the equations of Hill. Fig. 5 shows a graphic representation of the concentration and response curves. The response corresponds to the curve of probability of an efficient therapy, and the toxicity corresponds to the curve of probability of drug adverse reactions. In the lower-left corner of the curve, there is no sufficient drug present to produce an observable response. Subsequently, there is a modest change in serum concentration, which produces a considerable change in the response. These drugs are usually denominated "concentration-dependent" with respect to their activity. This is the case for quinolones, aminoglycosides, and rifampicins. When the concentrations reach a plateau (in the upper-right portion of the curve), the addition of drugs seem to produce no greater increases in the response. Drugs that operate in this part of the curve are usually called "concentration-independent" with respect to their activity. The toxicity profile dictates where on the curve we must operate. The separation between both curves along the *x*-axis defines

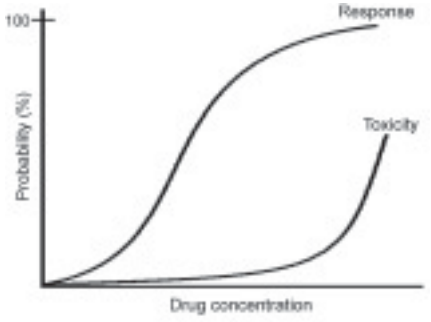

Fig. 5: graphical representation of the curves of concentration and response to drugs.

the "therapeutic range" of the drug. The maximum dose for any patient is the one that produces the desired therapeutic effect with an acceptable level of toxicity. However, when the disease reaches a life-threatening threshold to a patient, it might be necessary to accept an additional toxicity to attain cure (Peloquin 2001).

Vaccine development

The mammalian immunological system acts either by a cellular or humoral response, and although both responses involve helper T lymphocyte (Th) cells, the outcome of the immune response depends on which subclass is involved. Helper T lymphocytes are produced by two maturation pathways (Th-1 and Th-2) and are grouped according to cluster differentiation (CD4 and CD8) and secrete different cytokines. Th-1 type immune response involves $CD8^+$ and $CD4^+$ helper T lymphocytes and is cellular in nature; it acts against chronic diseases, such as intracellular parasitism and cancer, through macrophage activation, and detection and lysis of affected cells. This process leads to granuloma formation, which is the paradigm of protective immunity in intracellular diseases. In contrast, Th-2 type immune response involves different subsets of $CD8^+$ and $CD4^+$ T lymphocytes and is humoral in nature. It may be required to eliminate extra-cellular antigens and parasites. However, this response can also cause tissue destruction and necrosis, which represents immune response failure in many diseases. Cells of the Th-2 maturation pathway secrete cytokines such as IL-4, IL-5, IL-6, IL-9, IL-10, and TNF; these cytokines inactivate macrophage proliferation, and TNF causes tissue inflammation and necrosis when released at high levels. Antibodies are produced due to interaction between B cells and activated $CD4^+$ T lymphocytes. These antibodies can be effective in the neutralization of infectious agents, but are not able to eliminate all the infected cells, and, thereby, the host will depend on cellular immune response to protect itself from pathologies that initiate in the intracellular environment. Cellular immune response involves Th lymphocytes produced by the Th-1 maturation cell pathway. These cells secrete cytokines such as IL-2, IL-12, IL-15, INF-γ, lymphotoxins, and granulocyte macrophage colony stimulating factor, which activate macrophage (Labidi et al. 2001).

As an attempt towards human immunization, the first vaccines were produced aiming at protection against acute infections, where Th-2 type immune responses can be efficient. These vaccines were composed of a variety of crude antigens, including dead or attenuated cells, toxins, and other structural components derived from the pathogen (Grange et al. 1995). More recently, efforts have been focused on isolating and developing single antigens or epitopes into vaccines (Labidi et al. 2001). In order to have a long-lasting protective immunity, new vaccines should combine selected antigens with potent adjuvants, and stimulate the appropriate immunological pathway (Grange et al. 1995). The ideal mycobacterial adjuvant would be the one that induces an increase in Th-1 response and a concomitant inhibition of Th-2 response. The mycobacterial material is selected based on its lack of pathogenicity in humans and animals, and its capacity of association with mammal cells, being in the form of live or dead mycobacterial cells from strains derived from non-pathogenic species. The ideal vaccine would be the one that contains immunogens that induce the formation of granulomas without necrosis. The latter is the paradigm of protective immunity, as a sign of bacterial death and regression of the disease. TB immunity is local, which means that the consequences of a lesion (where the first invasive bacteria remained) depend on the nature of T cells that are attracted to the lesion. If the attracted immune cells are Th-2, there is a lesion-necrosis evolution and disease progression; if, however, the attracted cells are Th-1, the bacteria are destroyed, there is granuloma formation, and both lesion and disease suffer regression.

BCG is currently the most widely used vaccine in the world against TB. It has been administered to 2 to 3 billion people (since 1948) without serious complications, is of easy inoculation and can also be administered as an oral vaccine, requires a single immunization and can confer immunity for a long period, is a very efficient adjuvant for immunity induction, and has a low cost of production (Labidi et al. 2001). However, recent molecular analysis have revealed that genetic modifications formed substrains along time, and a complete avirulence acquisition is also suspected for the vaccine, making immunization impracticable. Another problem is that many years after BCG immunization, there can be a gradual loss of T memory cell population (Orme 2001). Since the efficacy of this vaccine varies from zero to 80% (Colditz et al. 1994), scientists are in a constant search for vaccines with some kind of modification, such as recombinant and other attenuated live mycobacterial strains. Although BCG has been effective to prevent meningeal TB in children, it does not confer protection to pulmonary TB in adults (Orme 2001). Thereby, it is necessary to turn BCG or mycobacterial molecules more immunogenic through the development of adjuvants, in order to induce a greater hypersensibility mediated by $CD4^+$ cells (Ehlers 1999). Even though BCG was originally developed for oral immunization, this delivery route was gradually replaced to its current intradermal use among most nations ever since the Lubeck (Germany) disaster, where a great number of children died from or developed active TB as a result of vaccine contamination with *M. tuberculosis*.

However, despite the great resistance, the Ataulpho de Paiva Foundation (Brazil) has retained efforts on research and production-distribution of the BCG Moreau Rio de Janeiro oral vaccine, as some physicians still recommend its use. Recent results of a phase I clinical trial have suggested that this strain is extremely immunogenic and causes negligible side effects as compared to any other BCG strain (Benevolo-de-Andrade et al. 2005). Anyhow, in order to develop alternative mycobacterial vaccines to BCG, it becomes necessary to identify all species of the *Mycobacterium* genera that can induce an appropriate immune response and be genetically favorable to cloning and expression of a wide spectrum of antigens. This requires a global knowledge and comprehension of the chemical structure of the cell wall and the genetics of each member of the genera (Labidi et al. 2001).

Currently, DNA vaccine is a widely explored area, where microbial DNA sequences can be used as target vaccines. The strategy consists on identifying immunogenic proteins, isolate its encoding gene, clone it into an expression vector that has a strong promoter, transform bacterial cells with the recombinant plasmid, and use the vector as a vaccine. Once injected into animal muscle cells, the plasmid is transcribed to RNA, and the cells express the recombinant protein (Orme 2001). DNA vaccines have used the mycolil-transferase Ag85 enzyme, which has demonstrated promising results in short and long term assays, and the hsp60 heat shock protein, which, when derived from *M. leprae*, showed to be effective in both prophylactic and immunotherapeutic form (Young et al. 1988, Lowrie et al. 1999), but when derived from *M. tuberculosis*, showed no protection, besides producing inflammatory reactions in the bronchi airways (Orme et al. 2001). However, since one third of the world population is infected with *M. tuberculosis*, two types of vaccines seem to be necessary: one to prevent the invasion of the pathogen (two thirds of the population) and the other to eradicate the already established infection (one third of the population) (Hess & Kaufmann 1999).

Perspectives

As the genome sequencing of *M. tuberculosis* H37Rv was completed, it was estimated that approximately 16% of its genome is dedicated to the codification of unknown proteins, which might be involved in specific mycobacterial functions, and can be interesting as potential drug targets and as antigens in vaccines. Strategies based on the discovery of new targets require the identification of specific biochemical pathways from mycobacteria and related organisms. Many of these metabolic processes occur during the biosynthesis of mycobacterial cell wall components, and some new attractive targets have appeared. The comprehension of the synthesis of the characteristic mycobacterial cell wall through the use of mycolic acids synthesis inhibitors can possibly lead to the development of new antimycobacterial agents.

The availability of three-dimensional structures of a biomolecule by X-ray diffraction crystallography and/or nuclear magnetic resonance spectroscopy introduces the possibility to design drugs based on a detailed model of the target binding site. Molecular modeling can also be employed to gather information for drug design. The rational design of new agents using computer assisted molecular modeling (CAMM), based on *M. tuberculosis* enzymes involved in essential biosynthetic pathways that are not present in humans is a promising alternative for anti-TB drug design. The currently available drugs used to fight TB were identified in the period of 1945 to 1967 by traditional prospection, that is, by chance. Considering that X-ray crystallography can determine a crystalline structure in nearly atomic resolutions for any protein of therapeutical interest, the detailed knowledge of the target protein both wild-type and mutant can assist in the design of a new ligand for the receptor binding site that blocks essential pathways in sensible and resistant strains.

Anyway, it is fundamental to perform the diagnosis of TB infected patients as soon and efficiently as possible in order to avoid active disease development and the dissemination of infective organisms into the population. It thus becomes extremely important to standardize the treatment method to which infected and/or actively ill patients should be submitted, as it is of vital priority to avoid the possibility of already established host infective bacteria to develop resistance to the drugs used in chemotherapy. We would like to suggest that a greater investment in the establishment of DOTS into all possible regions, as the development of drug-resistant mycobacterial strains in appropriately treated patients has been proven to be statistically improbable. Notwithstanding, identification of new targets and the development of new antimycobac-terial agents are urgently needed to combat the drug resistant strains of *M. tuberculosis*, and to strengthen the current treatment and to shorten the "short-course" treatment to improve patient compliance.

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