REVIEW

Revisiting the Natural History of Tuberculosis

The Inclusion of Constant Reinfection, Host Tolerance, and Damage-Response Frameworks Leads to a Better Understanding of Latent Infection and its Evolution towards Active Disease

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Abstract Once *Mycobacterium tuberculosis* infects a person it can persist for a long time in a process called latent tuberculosis infection (LTBI). LTBI has traditionally been considered to involve the bacilli remaining in a non-replicating state (dormant) in old lesions but still retaining their ability to induce reactivation and cause active tuberculosis (TB) once a disruption of the immune response takes place. The present review aims to challenge these concepts by including recent experimental data supporting LTBI as a constant endogenous reinfection process as well as the recently introduced concepts of damage-response and tolerance frameworks to explain TB induction. These frameworks highlight the key role of an exaggerated and intolerant host response against *M. tuberculosis* bacilli which induces the classical TB cavity in immunocompetent adults once the constant endogenous reinfection process has resulted in the presence of bacilli in the upper lobes, where they can grow faster and the immune response is delayed. This essay intends to provide new clues to understanding the induction of TB in non-immunosuppressed patients.

The Present Vision of *M. tuberculosis* Infection

Natural History of Tuberculosis

Mycobacterium tuberculosis infection starts with the phagocytosis of the bacilli by the alveolar macrophage. These bacilli can resist the bactericidal mechanisms induced by the macrophage (i.e. phagolysosome formation) and multiply inside the phagosome, thus avoiding macrophage apoptosis (Bermudez et al. 2006). Finally, they cause macrophage necrosis and thereby enter the extracellular milieu, where they are phagocytosed by another macrophage which also fails to control the bacillary growth and is likewise destroyed. This cycle ends once the specific immune response appears (Kaufmann et al. 2005) based on the production of Th1 CD4 T lymphocytes able to synthesize IFN-γ which recognize infected macrophages showing antigens from growing bacilli (Andersen 1997; Wolf et al. 2008). This is the end of the progressive infection. At this point, the bacilli enter a state of latency based on resistant bacillary forms capable of surviving under the stressful conditions generated by the host (Cardona 2007; Gill et al. 2009; Muñoz-Elias et al. 2005; Wallace 1961). Hypoxia has long been recognized as the main environmental condition for inducing the adaptive stage of bacilli, known as "dormancy", where the DosR operon plays a crucial role in increasing the expression of different genes involved in dormancy induction (Ulrichs and Kaufmann 2006; Wayne and Sohaskey 2001). These bacilli can inhabit old granulomas during the lifetime of the

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host and are able to resuscitate in the event of local immunodepression (Dannenberg 2006). In immunocompetent people, this reactivation usually takes place in the upper lobes, where the high oxygen pressure encourages bacillary growth (Gordon and Mwandumba 2008; Milic-Emili 2005; Park et al. 1992). In this location, a process of liquefaction occurs in the intragranulomatous necrosis (or caseum) triggered by the macrophages, the bacilli, or both, which favors the extracellular growth of bacilli and increases the inflammatory response. This process finally results in bronchial erosion and drainage of the liquefied material, which leads to the formation of a cavity in the lung. This factor is not that crucial in immunosuppressed hosts, where the reactivation that occurs in different locations and cavitation is less frequent due to the lack of a sufficiently strong inflammatory response (Gordon and Mwandumba 2008).

Treatment

Treatment of active tuberculosis (TB) is based on the assumption that four kinds of bacillary populations coexist in the cavity: one actively growing population, two located intracellularly inside macrophages or in an extracellular site with a low pH and characterized by starting growth spurts (Grosset 1980; Mitchison 1979), and a fourth population of non-replicating bacilli which, having no metabolic activity, are impossible to destroy by chemotherapy. As there is active extracellular growth in the liquefied necrotic tissue generated inside the lesion, the bacillary concentration is high enough to generate spontaneous resistance mutations to the different drugs given individually (Grosset 2003). This is why at least three drugs are given in the first treatment phase. In the second phase, only two antibiotics are given to kill non-replicating bacilli that are trying to grow. Although no clear statement has been made so far, isoniazid treatment of latent tuberculosis infection (LTBI) can also be explained to avoid regrowth of non-replicating bacilli in old lesions. Considering that this reactivation is likely to take place during the lifetime of the host, it is hard to believe that a nine-month treatment consisting of only one drug will be able to kill growing bacilli effectively (Cardona 2007; Cardona 2009).

New Concepts that Challenge the Present Vision on *M. tuberculosis* Infection

The Importance of the Life Cycle of Bacilli

Although considerable information has been gathered regarding the nature of *M. tuberculosis*, including its metabolism, its antigen profiles during growth periods, its

ability to respond to stressful conditions, and its interaction with the different cytoplasmic structures of macrophages, this knowledge has yet to be used to study the response generated by the host in the infection process or integrated into cellular biology studies. Such studies are usually run only in log-phase bacilli (Parish and Stoker 2001). This is worth mentioning because nowadays we have some information regarding the survival capacity and antigenic presentation of actively growing bacilli, which are only compared with the killed, whereas little is known about those that are in a stationary phase. Furthermore, there is no detailed understanding of the growth capacity of bacilli. It is known that *M. tuberculosis* grows intracellularly in the host and can only grow extracellularly in an optimum environment, such as the liquefied necrosis inside the cavity (Grosset 2003). This fact, however, is usually overlooked or not strongly affirmed. The perfect environment for *M. tuberculosis* is inside phagosomes, and once outside these structures it stops growing and enters a
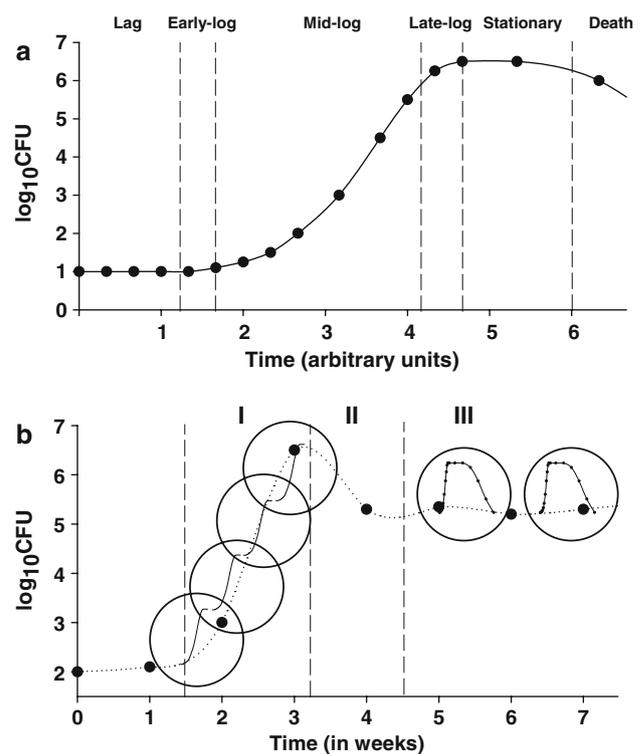

Fig. 1 Hypothesis concerning the evolution of *M. tuberculosis* bacillary growth in a human host (based on Cardona 2007). **a** Classical in vitro bacterial life cycle in a culture with limited nutrients, according to Buchanan (Buchanan 1918). **b** The kinetics of the bacillary concentration in the experimental murine model, including the theoretical dynamics of the growing bacilli and taking into account the life cycle of the bacteria (represented inside the circles). An exponential growth can be distinguished (*I*), a bacillary control induced by the specific immunity that induces the destruction of 90% of the bacillary bulk (*II*), and the chronic phase, in which the bacillary concentration remains stable (*III*)

stationary phase (Fig. 1), eventually becoming non-replicating (Cardona 2007). This means that there is a constant cycle of log versus stationary phases from the very early stages of the infection process rather than just at the end as a result of pressure caused by the hypoxia generated in old granulomas (Wayne and Sohaskey 2001). In this scenario, the bacilli have to adapt constantly to different environments. The extracellular milieu contains numerous bactericidal enzymes from macrophages and neutrophils that have been destroyed, and it becomes acidic and hypoxic because the reorganized capillary net is not sufficiently efficient (Martinez et al. 2007; Fukumura and Jain 2008). Neutrophils accumulate at the start of the infection and have a very short lifetime, which means that these dead cells accumulate and release their cytoplasmic content (Fig. 2). All these factors induce the presence of non-replicating bacilli even before the immune response has been triggered. As non-replicating bacilli are better able to resist stressful conditions, not all bacilli are killed once the immune response allows activation of the infected macrophages. It therefore appears logical that only the actively growing bacilli population is killed when this activation occurs. The majority of non-replicating bacilli are therefore able to resist the bactericidal effect induced by the activation (Wallace 1961).

Foamy Macrophages and the Constant Reinfection Process

An apparently useless kind of cell appears in this scenario: the foamy macrophage. As in any other chronic inflammatory process with high levels of cellular destruction, macrophages tend to phagocytose the remaining cellular debris (Dvorak et al. 1983; D'Avila et al. 2006). As cell membranes are rich in fatty acids and cholesterol, these deposits become organized into lipid bodies in the macrophage cytoplasm. This process is even more significant if a large necrotic area exists in the lesion. The necrosis is organized with fibrin and collagen fibers where the extravasation of plasma from capillaries accumulates low-density lipoprotein (LDL) particles. Once oxidized by the free radicals present in the granulomas, oxidized LDL particles are also phagocytosed by the macrophages that survey this area, thereby further increasing the quantity of intracytoplasmic lipid bodies (Murphy 2001). These lipid bodies eventually tend to accumulate cholesterol and form crystals, which can increase in size to such an extent that they destroy the macrophages. This process is very well known and studied in atherosclerosis (Ross 1999).

In *M. tuberculosis* infection, foamy macrophages also phagocytose the extracellular non-replicating bacilli
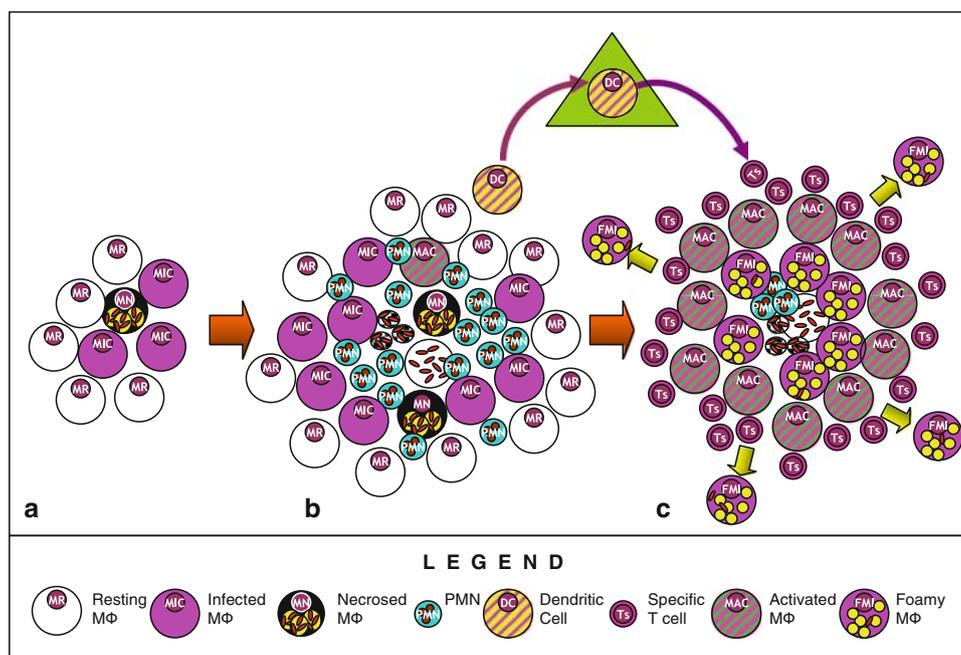

Fig. 2 Recreation of the evolution of the infection. **a** Necrosis of an infected alveolar macrophage (MN), which releases extracellular bacilli that are phagocytosed by the remaining macrophages (MR) and themselves become infected (MIC). **b** The progression of this granuloma, showing an accumulation of neutrophils, a minimal proportion of activated macrophages (MAC) because of innate factors, and the induction of dendritic cells (DCs). These DCs migrate towards the lymphatic nodes (*green triangle*) where, once they reach a certain concentration, they stimulate the formation of specific lymphocytes (*Ts*) that are able to activate infected macrophages. **c** A granuloma where specific lymphocytes are present and foamy macrophages (*FM*) become visible
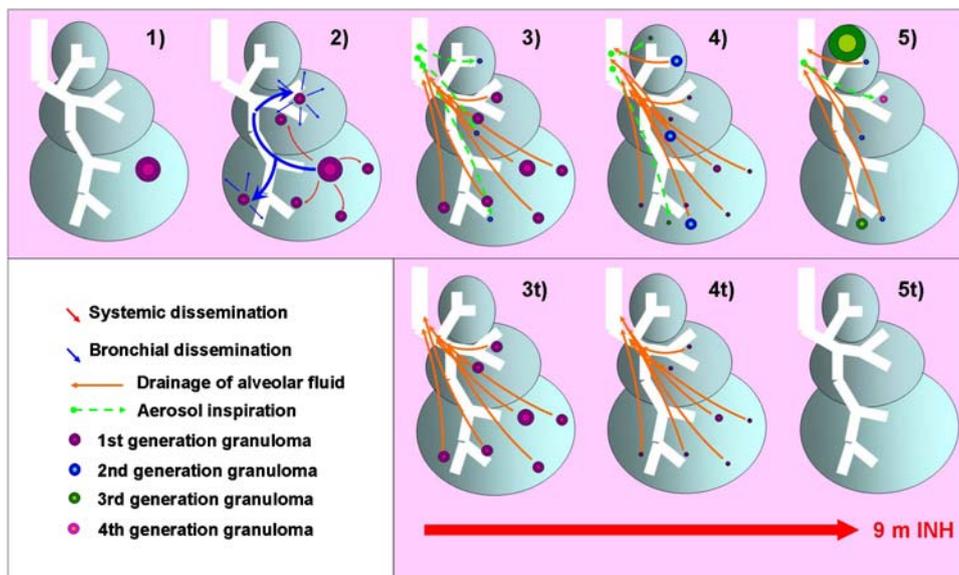
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Fig. 3 Latent tuberculosis infection and the generation of active TB according to the dynamic hypothesis (based on Cardona 2009). Once the initial lesion has been generated (*1*), bronchial (*blue arrows*) and systemic (*red arrows*) dissemination generate new secondary granulomas (*2*). This process is stopped once the specific immunity has been established, which starts a constant drainage of non-replicating bacilli towards the bronchial tree (*solid arrows*) to which the inspired aerosols (*dotted arrows*) can return, thereby generating new granulomas (*3, 4*). This process implies finding different generations of granulomas simultaneously. In this dynamic process, if one of these reinfections takes place in the upper lobes, it will have the opportunity to induce a cavitary lesion (*5*). Isoniazid treatment (*3t–5t*) allows the drainage of the non-replicating bacilli, preventing them from reactivating. In this case the lesions disappear with time and the possibility of reaching the upper lobes and generating the cavitary lesion is avoided

(Caceres et al. 2009; Cardona et al. 2000; Cardona et al. 2003; Peyron et al. 2008). Despite being in an intracellular milieu, these bacilli do not grow again, probably because all the macrophages have already been activated. In this scenario, the non-replicating bacilli tend to accumulate fatty acids and generate intracellular lipid bodies, which is also related to their stationary condition (Garton et al. 2002; Garton et al. 2008). Interestingly, these foamy macrophages leave the parenchyma through the alveolar space, forming part of the alveolar fluid which is constantly generated to remove all the toxic particles carried by the inspired air (Caceres et al. 2009; Cardona et al. 2000; Cardona et al. 2003; Peyron et al. 2008). This inspiration process is mainly responsible for generating aerosols (Bui et al. 1998), which is why we have hypothesized that the non-replicating bacilli that are constantly drained from the lung granulomas can be carried by these aerosols, thereby returning to the parenchyma and infecting it once again (Cardona 2009). This is the only hypothesis that can explain the success of treating LTBI with isoniazid for only nine months (Cardona 2009). According to the traditional theory, non-replicating bacilli can remain in an old lesion during the lifetime of the host (Ulrichs and Kaufmann 2006), which means that treatment with a drug which only kills actively growing bacilli cannot be efficient unless a constant reinfection process is taking place. In this case, non-replicating bacilli would be drained and destroyed in

the stomach and isoniazid would prevent reinfection. This mechanism would therefore explain the actual success of the present gold-standard treatment (Fig. 3).

Active TB Induction

Damage-Response Framework

It is widely accepted nowadays that both the microorganism and the host contribute to microbial pathogenesis (Casadevall and Pirofski 2003). *M. tuberculosis* infection is well characterized in the Group 3 pathogen class and can produce damage at both ends of the continuum of immune responses (Fig. 4). It is clear that a lack of host response favors bacillary growth and leads to host death. In fact, all the present strategies for confronting *M. tuberculosis* infection tend to focus on understanding which immunological disorder leads to the disappearance of the immune response in LTBI, which should, in turn, lead to the induction of active TB.

It appears, however, that no authors are interested in the other end of the scale, where a too strong host response also leads to disease. It is true that some authors have, in the past, focused on both sides of the same protective immunity, namely cell-mediated immunity (CMI) and delayed type hypersensitivity (DTH) (Dannenberg 2006). Weaker hosts tend to generate DTH because they are not strong
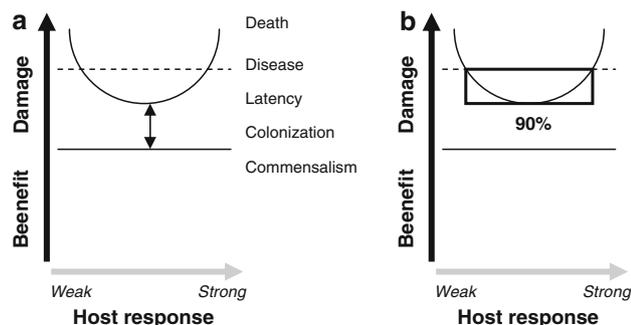
Fig. 4 Evolution of TB according to the damage-response framework (based on Casadevall and Pirofski 2003). **a** The y-axis shows the progression of host damage and the x-axis the intensity of the host response. The *arrow* indicates that the position of the curve is variable and depends on each particular host-microorganism interaction. Weak responses are those considered insufficient, poor, or inappropriate (i.e. not strong enough to benefit the host), whereas strong responses are those that are excessive, overly robust, or inappropriate (i.e. too strong, thereby damaging the host). **b** represents one hypothesis to explain the origin of active TB. In brief, 90% of humans have a balanced response that permits them to avoid disease

enough to induce CMI, and this leads to the destruction of the infected macrophages. Classical studies with resistant and susceptible rabbits were mainly performed to solve this question. Susceptible rabbits tended to generate more necrosis than resistant ones because of the predominance of DTH over CMI. Interestingly enough, however, only the resistant ones were able to induce cavitation, which might not seem coherent (Lurie 1964; Dannenberg 2006). The authors explained this observation by postulating that cavitation was a way to drain the bacilli, something that only resistant rabbits can do (Doenhoff 1998). Somewhat surprisingly, necrosis and liquefaction were not considered to be linked in that model.

Since then, immunologists have tried to explain the induction of active disease as a means of reducing the cellular immunity generated by Th1 cells. This decrease has been consistently related to the presence of the Th2 response, which is a more humoral response that counterbalances Th1 and is mostly related to the induction of

necrosis and the presence of active TB (Rook 2007). To date, nobody has been able to demonstrate whether, by counterbalancing Th1, Th2 is the cause of cavity induction or if it is induced once cavitation has taken place, as the presence of a high burden of extracellular bacilli requires a humoral response to control it.
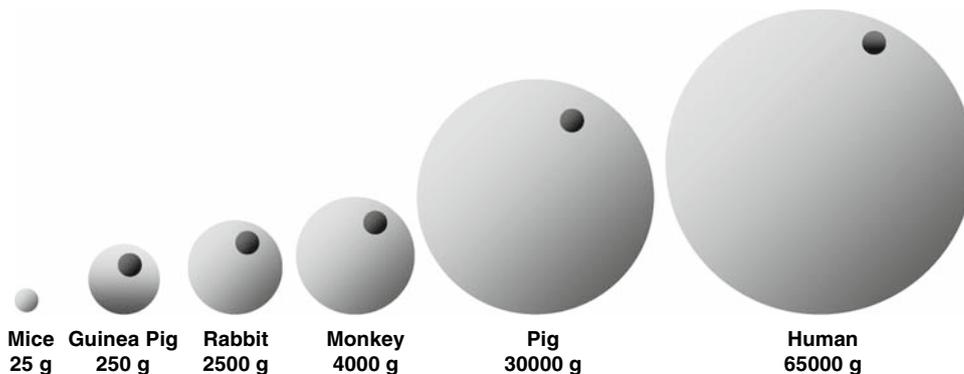
Tolerance and Resistance

Some years ago, we postulated against the traditional concept related to two further experimental TB models, namely those induced in mice and guinea pig (GP), which were resistant and susceptible, respectively (Dannenberg 2006, Orme and Gonzalez-Juarrero 2007). Our proposal was based on the belief that infection in mice does not seriously threaten their survival, whereas guinea pigs die rapidly. Interestingly, looking at the colony forming units (CFU) and histological aspects, mice do worse than GPs as they induce a poorly granulomatous structure which allows systemic dissemination, whereas GPs induce a human-like granulomatous response, with well-structured granulomas which delay the systemic dissemination that finally kills the animal (Dannenberg 2006, Cardona 2006).

While searching for new methods of therapeutic vaccination, we hypothesized that mice should probably be considered a "tolerant" host as they allow the presence of high CFU concentrations, whereas GPs do not and are finally killed because of the strong inflammatory response induced. At that point we postulated the "volume theory", which supports the idea that mice can never generate a cavity because it would cause their instant death (Cardona 2006) (Fig. 5). This is why we highlighted the need to work on experimental models in hosts with larger volumes in order to better understand what happens in humans, especially the induction of toxicity after a therapeutic vaccination (Cardona 2006).

Some authors have recently included the concepts of "tolerance" and "resistance", which are widely used in the plant ecology community, in infectious diseases (Ayres and Schneider 2008; Schneider and Ayres 2008). These authors

Fig. 5 Theory of volume and inflammatory response (based on Cardona 2006). Small hosts tend to trigger a lower inflammatory response to maintain their body integrity. Mice will never generate a human-like TB cavity because it would quickly kill them. The picture compares different hosts according to their weight. The volume of a TB cavity weighing 25 g is shown in *dark grey*
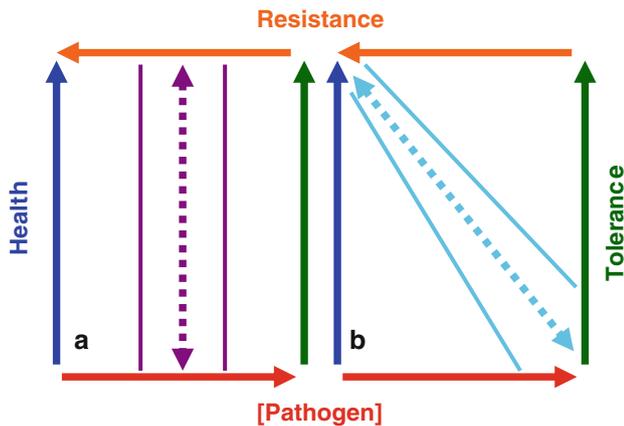
Mice **25 g**	**Guinea Pig** **250 g**	**Rabbit** **2500 g**	**Monkey** **4000 g**	**Pig** **30000 g**	**Human** **65000 g**

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Fig. 6 Integration of the tolerance and resistance concepts to infectious diseases (based on Ayres and Schneider 2008). **a** Variation of the degree of tolerance directly affects the health status of the host but has no effect on the pathogen concentration. On the other hand, a decrease in the degree of resistance diminishes the health status and also causes an increase in the pathogen load (**b**)

consider tolerance to be the ability to tolerate a certain concentration of pathogen without the health of the host being affected, whereas resistance is related to the ability to control the pathogen concentration (Fig. 6). Going back to our previous hypothesis, humans would not tolerate the presence of the bacilli at all; therefore controlling the bacillary load induces a very strong response which leads to a toxic effect, larger granulomas, and a stronger necrotic reaction than in smaller animals such as mice, which show a weaker response and thereby allow the presence of high bacillary concentrations (Fig. 7).

Endpoint: How Active TB Takes Place? Damage, Tolerance, and Virulence

Bringing all these ideas together, we hypothesize that the history of TB is mainly based on a process of constant endogenous reinfection that allows the bacilli to persist in the host for a long time. This control is highly effective in humans, with no active disease occurring in around 90% of cases. If we ignore the endpoint postulated by the theory whereby damage is caused by an immunosuppressive event, this efficacy is probably higher. As humans are highly non-tolerant, once the presence of a small quantity of bacilli is identified, a huge inflammatory response that quickly prevents bacillary growth and dissemination is triggered. This can clearly be seen in experimental models run in high-volume hosts (such as cows and dwarf pigs) in very small granulomas (<1 mm) characterized by a predominance in the necrotic tissue (Bolin et al. 1997; Buddle et al. 2005; Gil et al. 2009).

The fact that TB in immunocompetents is mainly induced in the upper lobes means that this site must be a target for the bacilli, which show a tropism for this location. It is easy to hypothesize that once this site has been reinfected, the bacilli grow quickly and the immune response undergoes some kind of delay. It has been postulated physiologically (regarding the poor capillary net and the increased oxygen pressure) (Park et al. 1992; Milic-Emili 2005; Gordon and Mwandumba 2008) that once the inflammatory response appears, it is so exaggerated because the bacillary concentration, although still low, is much higher than the levels tolerated by humans. This means that the immune response subsequently triggered is so strong that it leads to mass tissue destruction which is impossible to structure with fibrin and collagen, thus leading to liquefaction and subsequent extracellular bacillary growth, which increases the response until it is overwhelmed and causes a cavitation. This process has some parallels with the sudden increase in CD4 T cells and the induction of the immune reconstitution inflammatory syndrome in AIDS patients receiving highly active antiretroviral treatment (Lipman and Breen 2006). In this case, even if harboring an *M. tuberculosis* concentration in their tissues, the presence of the bacilli is well tolerated because the host is unable to trigger the inflammatory response required to control their growth. When this immune response suddenly appears thanks to the antiretroviral therapy, it must face a high bacillary concentration, which means that the granulomatous response is too aggressive and causes disease.

In this regard, it can be hypothesized that the virulence of *M. tuberculosis* strains (understood as the ability to generate active disease) could be related to their ability to change promptly from a non-replicating to a replicating state in order to grow as fast as possible in the upper lobes.

Conclusion

This work reflects a philosophical problem to some extent, namely that immunity against *M. tuberculosis* in humans is already highly efficient and therefore difficult to improve. It is thus difficult to separate the toxic from the beneficial response for it to be even more efficient. In other words, the immune response cannot be tolerant only sometimes, which would avoid the induction of pathogenesis at the strong-response end of the damage-response framework. It could be postulated that the majority of the population has a balanced response that makes them not particularly non-tolerant, i.e. they have a medium-strength immune response (Fig. 4). On the other hand, it can also be postulated that all humans have the same degree of non-tolerance and it is reinfection of the upper lobes which
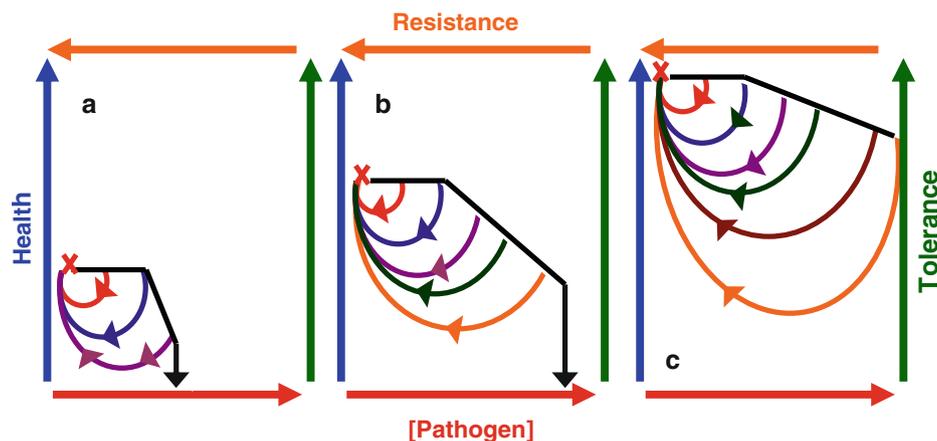
Fig. 7 Adaptation of the tolerance-resistance framework to *M. tuberculosis* infection. The three pictures show hosts with different degrees of tolerance. The initial load of bacilli is shown with a *red times* and the *curves* show different cases in the natural history. The *red curve* represents the original infection, where a lack of initial immunity allows the bacilli to progress quickly until immunity appears and blocks bacillary growth. The immune response also has a component of intolerance that causes a small amount damage to the host until the bacillary load is controlled and the damage fixed. The *blue curve* represents the induction of a cavity. In this case, as they are in a privileged place (the upper lobe), the bacilli grow without causing any health effects until the host response, with its dual component of damage and bacillary control, appears. From this point, it is considered that the bacillary increase always causes some damage, which will depend on the degree of host tolerance. Finally, the host tries to counterbalance this bacillary progression and to reestablish its health; the more tolerant the host, the better its chance of surviving as it has more chance to control the bacillary load without impairing too much its health status. Beyond the *blue curve* there are others which coincide with different immunosuppressive stages that allow a higher bacillary load, thereby influencing the health status according to the general degree of tolerance of the host, but which show no resistance. The two extreme examples of tolerance would be humans on one side (**a**) and mice on the other (**c**)

finally results in active TB. This would reduce the problem to a mechanistic matter related to the chances of the bacilli reaching the upper lobes. The actual response is probably somewhere in the middle, although this approximation unfortunately leads to a rather complex and technically difficult scenario: to look for a balanced, somewhat "tolerant", immune response.

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