Special Communication

Modelling of Cerebral Tuberculosis: Hope for Continuous Research in Solving the Enigma of the Bottom Billion's Disease

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Abstract

Cerebral tuberculosis is a severe type of extrapulmonary disease that is highly predominant in children. It is thought that meningeal tuberculosis, the most common form of cerebral tuberculosis, begins with respiratory infection followed by early haematogenous dissemination to extrapulmonary sites involving the brain. Host genetic susceptibility factors and specific mycobacteria substrains could be involved in the development of this serious form of tuberculosis. In this editorial the different animal models of cerebral tuberculosis are commented, highlighting a recently described murine model in which BALB/c mice were infected by the intratracheal route with clinical isolates, which exhibited rapid dissemination and brain infection. These strains were isolated from the cerebrospinal fluid of patients with meningeal tuberculosis; they showed specific genotype and induced a peculiar immune response in the infected brain. This model could be a useful tool to study host and bacilli factors involved in the pathogenesis of the most severe form of tuberculosis.

Keywords: *experimental models, infectious diseases, meningeal tuberculosis, mice, Mycobacterium, virulence*

Tuberculosis and the Central Nervous System

Tuberculosis involvement of the central nervous system (CNS) is a significant and serious type of extrapulmonary disease. It constitutes approximately 5%–15% of the extrapulmonary cases, and in developing countries, it has high predominance in children (1). There are different clinical/pathological manifestations of cerebral tuberculosis; the most common is tuberculous meningitis, followed by tuberculoma, tuberculous abscess, cerebral miliary tuberculosis, tuberculous encephalopathy, tuberculous encephalitis, and tuberculous arteritis (2). Cerebral tuberculosis is often fatal and mainly caused by *Mycobacterium tuberculosis*; other non-tuberculous mycobacteria such as *M. avium-intracellulare* can also produce CNS tuberculosis, mainly in human immunodeficiency virus (HIV)-infected persons (2).

It is believed that cerebral tuberculosis, like any other forms of tuberculosis, begins with respiratory infection followed by early haematogenous dissemination to extrapulmonary sites, including the CNS. On the basis of their clinical and experimental observations, Rich and McCordock (3) suggested that cerebral tuberculosis develops in two stages. Initially, small tuberculous lesions (Rich's foci) develop in the brain during the stage of bacteraemia of the primary tuberculosis infection or shortly afterwards. These early tuberculous lesions can be located in the meninges, the subpial or subependymal surface of the brain, and may remain dormant for long time. Later, rupture or growth of one or more of the small lesions produces development of various types of CNS tuberculosis. Rupture into the subarachnoideal space or into the ventricular system produce meningitis, the most common form of cerebral tuberculosis.

Modelling of Cerebral Tuberculosis

Experimental animal models of cerebral tuberculosis have been established in rabbits (4,5), mouse (6,7), and pigs (8). Although they reproduce in some extend the human lesions, these models are artificial because they use the direct intracerebral or intravenous route of infection, instead of the natural respiratory route. Thus, it is important to establish an experimental

model which reproduces more closely the human disease, including the initial respiratory natural route of infection. However, such model is difficult to achieve because of the highly efficient CNS protection conferred by the blood-brain-barrier (BBB). BBB is composed of tightly associated brain microvascular endothelial cells covered by pericytes and outgrowths of astrocytes (cytoplasmic end feet). This structure efficiently prevents CNS infection by many microorganisms, including mycobacteria. Thus, to produce CNS infection, some microorganisms have evolved specific virulence factors that permit, first, endothelial attachment and internalization, followed by brain parenchyma invasion (9). This is the case of bacterial proteins IbeA, IbeB, AslA, YijP, and OMPA expressed by neurotropic *Escherichia coli*, or meningococcal surface proteins Opa, Opc, and PiIC among others.

Recent in vitro studies have shown that *M. tuberculosis* can adhere, invade, and traverse endothelial cells (10), and clinical–epidemiological studies have shown distinct genotype in strains isolated from tuberculous patients' cerebrospinal fluid (CSF) (11), which suggest strain-dependent neurovirulence and neurotropism. We recently informed the results from an experimental study in which, using a model of pulmonary tuberculosis in BALB/c mouse infected by the intratracheal route, three different *M. tuberculosis* clinical isolates obtained from CSF of meningeal tuberculous patients were able to rapidly disseminate and infect the mouse brain (12). These clinical strains were isolated from patients with meningeal tuberculosis in Colombia, and they showed a distinctive genotype. They extensively disseminated by haematogenous route after one day of intratracheal infection and rapidly produced tuberculous lesions in the mice brain. As mentioned before, it has been established that mycobacteria reach the CNS by the haematogenous route secondary to pulmonary infection (3). This experimental model is the first one that reproduced this situation, confirming that the strain type is directly related with the ability to disseminate by the haematogenous route, and add *M. tuberculosis* to the list of microorganism families in which some members or substrains have certain ability to infect the CNS.

Comprehensive clinical-epidemiological studies have identified several risk factors for meningeal tuberculosis; these include age less than 40 years, (HIV) infection, and certain ethnic populations. The latter factor suggests significant interplay between host genetic background and specific strains of mycobacteria (11). In fact, it has been recently shown association between the development of tuberculous meningitis and single nucleotide polymorphism in the Toll-interleukin-1 receptor domain containing adaptor protein (TIRAP) and Toll-like receptor (TLR-2) genes (13).

Our strains are from the Euro-American lineage which was recently reported to be more pulmonary than meningeal. It has been proposed that Euro–American strains are less capable of extra pulmonary dissemination due the lack of pks 15/1 intact gene. Pks gene participates in the production of phenolic glycolipid (PGL), which inhibits the innate immune response and may be responsible for dissemination and CNS infection. Our Euro–American isolates are unable to express PGL; however they efficiently disseminate and produced brain infection, suggesting that other mycobacterial molecules could participate in this process. We consider mycobacterial heparin-binding haemaglutinin adhesin as a good candidate because this molecule triggers receptor-mediated bacilli adherence and invasion to epithelial cells, and extrapulmonary dissemination. Another potential participating molecule is histone-like protein (HLP), which permits to *M. leprae* interacts with laminin on the surface of Schwann cells, facilitating its invasion. HLP is also expressed by *M. tuberculosis*, and could participate in the infection of the nervous cells after endothelial barrier traverse. This study is now in progress in our laboratory.

The first step required for certain neurotropic microorganisms to cause CNS infection is to penetrate the BBB. The basic and first element of BBB is microvascular endothelial cells that differ from those in other tissues by tight junctions with high electrical resistance and a relatively low number of pinocytotic vesicles. Recently, an in vitro model using human brain microvascular endothelial cells showed that the reference strain *M. tuberculosis* H37Rv can invade and traverse these cells, using a process that requires active cytoskeleton rearrangements. By microarray expression profiling, the authors found 33 genes that were overexpressed during endothelial cells invasion, suggesting that the products of these genes might participate in this process (10). Perhaps different gene profile could be expressed by our strains than the laboratory reference strain H37Rv used in the in vitro system that our experimental model showed limited ability to infect the brain.

Intravenous infection of C57Bl mice with *M. avium* induces brain infection, and the number of bacteria increases with the duration and level

of bacteraemia, which depend of the inoculum size (7). One of our strains (code 209) was highly virulent; it induced more rapid animal death and high bacilli burdens in the lung, liver, spleen, and particularly in the blood. In order to study if hypervirulence could be related to dissemination and brain infection, we infected animals with other highly virulent strains isolated from the sputum of patients with pulmonary tuberculosis. Interestingly, these pulmonary strains produced minimal brain infection, suggesting that hypervirulence is not always related with the ability to CNS infection. Moreover, infection of mice with the other CSF-isolated strains (codes 136 and 28), which efficiently infected the brain, produced higher or similar mice survival and bacilli loads than infection of mice with mild, virulent pulmonary strains.

The most distinctive histological features were small or middle size nodules constituted by lymphocytes and macrophages located in the cerebral parenchyma near to piamadre or below the ependimal cell layer (Rich's nodules). During late infection these nodules were larger and connected with the subarachnoideal space producing mild or extensive inflammatory infiltrate in meninges. Another interesting histological observation was the presence of extracellular positive acid fast bacilli or intracellular in astrocytes or microglia cells without inflammatory response. Immunohistochemical detection of mycobacterial antigens showed strong positivity in activated microglia, astrocytes, ependimal cells, and meningothelial cells, indicating that all these cell types are able to phagocytose mycobacteria or their debries. Indeed, under physiological conditions, basal expression of significant innate immunity receptors involved in mycobacteria phagocytosis (such as TLR-2 and TLR-4) were detected in the meninges, choroids plexus, and circumventricular brain area, which lack BBB and are more exposed to pathogens. Microglia and astrocytes can also express these receptors. Thus, in brain mycobacterial infection specific bacterial antigens and their host cell receptors, as well as innate immunity receptors are involved.

In many areas where we found strong mycobacterial-antigen immunostaining in CNS cells, there were not inflammatory infiltrate. Thus, an efficient modulation of inflammation is produced in the brain which could avoid or delay tissue damage and signs of neurological lesion, even in the presence of high amount of bacilli. Th-2 cells could participate in this process, due to its efficient activity to suppress Th-1 high activity that induces excessive inflammation and tissue damage. Indeed, the Th-2 response also has beneficial activity for CNS, favouring healing and supporting neuronal survival. We found progressive IL-4 expression in the brain of mice infected with either of the three different clinical strains isolated from the CSF of meningeal tuberculous patients, while reference strain H37Rv induced low expression of Th-2 cytokine. Another significant anti-inflammatory cytokine localized in activated microglia is transforming growth factor beta (TGFβ). We found high gene expression of TGFβ in the brain of mice infected with CSF clinical isolates, and our histological studies showed positive immunostaining in macrophages located in Rich's nodules, as well as in activated microglia and capillary endothelial cells from distant areas of the inflammatory response. Thus, neuroprotection and suppression by specific cytokines should be a significant factor to avoid excessive inflammation and tissue destruction in mycobacterial CNS infection. This is in agreement with the observation that during late infection, when high bacilli loads in the brain were determined, none of the infected mice developed evident clinical signs of neurological damage, such as seizures or paralysis. The same situation has been reported in mice infected with high doses of *M. avium* by the intravenous route (7), or even in mice infected directly in the brain (6). However, we observed significant histological damage in the hippocampus area, where many neurons showed acidophilic necrosis and extensive gliosis in completely absence of inflammation. These histological abnormalities should be related with memory and cognocitive disturbances.

We consider that this experimental model, which demonstrates for the first time the existence of apparently neurotropic mycobacterial strains, could be a useful tool to study host and bacilli factors involved in the pathogenesis of the most severe form of tuberculosis.

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References

1. Garg RK. Tuberculosis of the central nervous system. *Postgrad Med J*. 1999;**75(881)**:133–140.
2. Katti MK. Pathogenesis, diagnosis, treatment, and outcome aspects of cerebral tuberculosis. *Med Sci Monit*. 2004;**10(9)**:RA215–229.
3. Rich AR, McCordock HA. The pathogenesis of tubercular meningitis. *Bull John Hopkins Hosp*. 1933;**52**:5–13.
4. Tsenova L, Sokol K, Freedman VH, Kaplan G. A combination of thalidomide plus antibiotics protect rabbits from mycobacterial meningitis-associated death. *J Infect Dis*. 1998;**177(6)**:1563–1572.
5. Tsenova L, Bergtold A, Freedman VH, Young RA, Kaplan G. Tumor necrosis factor alpha is a determinant of pathogenesis and disease progression in mycobacterial infection in the central nervous system. *Proc Natl Acad Sci USA*. 1999;**96(10)**: 5657–5662.
6. van Well GT, Wieland CW, Florquin S, Roord JJ, van der Poll T, van Furth AM. A new murine model to study the pathogenesis of tuberculous meningitis. *J Infect Dis*. 2007;**195(5)**:694–697.
7. Wu HS, Kolonoski P, Chang YY, Bermudez LE. Invasion of the brain and chronic central nervous system infection after systemic Mycobacterium avium complex infection in mice. *Infect Immun*. 2000;**68(5)**:2979–2984.
8. Bolin CA, Whipple DL, Khanna KV, Risdhal JM, Peterson PK, Molitor TW. Infection of swine with Mycobacterium bovis as a model of human tuberculosis. *J Infect Dis*. 1997;**176(6)**:1559–1566.
9. Huang SH, Jong AY. Cellular mechanisms of microbial proteins contributing to invasion of the blood-brain barrier. *Cell Microbiol*. 2001;**3(5)**:277–287.
10. Jain SK, Paul-Satyassela M, Lamichhane G, Kim KS, Bishai WR. Mycobacterium tuberculosis invasion and traversal across an in vitro human blood-brain barrier as a pathogenic mechanism for central nervous system tuberculosis. *J Infect Dis*. 2006;**193(9)**:1287–1295.
11. Arvanitakis Z, Long RL, Herschfield ES, Manfreda J, Kabani A, Kunimoto D, et al. M. tuberculosis molecular variation in CNS infection: Evidence for strain-dependent neurovirulence. *Neurology*. 1998;**50(6)**:1827–1832.
12. Hernandez Pando R, Aguilar D, Cohen I, Guerrero M, Ribon W, Acosta P, et al. Specific bacterial genotype of Mycobacterium tuberculosis cause extensive dissemination and brain infection in an experimental model. *Tuberculosis (Edinb)*. 2010;**90(4)**:268–277.
13. Hawn TR, Dustan SJ, Thwaites GE, Simmons CP, Thuong NT, Lan NT, et al. A polymorphism in Toll-interleukin 1 receptor domain containing adaptor protein is associated with suceptibility to meningeal tuberculosis. *J Infect Dis*. 2006;**194(8)**:1127–1134.