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Cutting edge in T.B. Research

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Abstract
TB is ancient disease. 50 million people are suffering from TB.10 Million people with positive sputum. 5, 00,000 deaths /every year. It is Gram positive Non Motile, non-sporing acid fast bacilli it spreads by cough, laugh, talking but not transmitted by kiss. For centuries, tuberculosis was a major killer disease, but the introduction of streptomycin in the late 1940s followed by isoniazid and in 1960s of rifampin, and ethambutol, revolutionized therapy, and tuberculosis came to be regarded as easily treatable condition. About one-third of HIV-associated deaths are caused by TB. The disease is out of control in many countries and is now the world's leading cause of death from a single agent. Mycobacterium tuberculosis, is the etiology agent of tuberculosis, the dread disease called consumption in Dickens time. One of the oldest and most devastating of human afflictions, tuberculosis remains a leading cause of infectious deaths worldwide today. From an ancient texts and historic documents, Medical historians have uncovered a wealth of information about the history of the disease. Now these written records are being supplemented by powerful new methods for studying impact that diseases have had on people and populations in the past. One of these new methods is ancient DNA analysis, writes Professor of Molecular Archeology Terry Brown.

Keywords: tubercle bacillus, mycolic acids, L.J. media, NKT cells, aminoglycoside, pyrazinamide, ethambutol, streptomycin, β-ketoacyl-ACP synthase (KasA), rifampin, BCG (Bacillus Chalmette–Gue´rin) vaccine

Introduction
In 2015, 10.4 million people were diagnosed with tuberculosis (TB) worldwide and 1.5 million died. Only 8 out of the 30 high-burden countries have achieved the World Health Organization (WHO) minimum target TB treatment success rates of 90% [1] Several factors may contribute to this low success rate including insufficient absorption, drug-drug interactions and other factors [2, 3] Tuberculosis (TB) remains a major public health problem in resource-poor countries including India. Scientific knowledge is used to guide policy and practice. [4] Some species of the Mycobacterium tuberculosis complex (MTBC), particularly Mycobacterium tuberculosis, are the first cause of death linked to a single pathogen worldwide. [5] Very little progress has been made in understanding the late stages that produce most disease and transmission of infection [6]. This is increasingly recognized as a major obstacle to development of new vaccines and host directed therapies [7, 8]. GTB is now undergoing a worrying recrudescence. So the disease needs renewed interest. [9] Tuberculosis is a hypersensitive granulomatous infectious disease caused by Mycobacterium Tuberculosis (M.TB) [10] Endometrial TB is fairly high in gynecologic patients visiting outpatient departments for various complaints and PCR detects more cases than culture or Histopathology. [11] The side effects associated with the allopathic drugs have remarkably necessities the need of herbal drugs. [12] Testicular tuberculosis (TB) is a rare form of genitourinary TB. It is usually presented as painful or painless testicular swelling with or without scrotal ulceration or discharging sinus. Infertility may occur. Epididymal involvement is usually seen in testicular TB. [13] The lack of specific biomarkers hinders these efforts. This study's purpose was to screen immunological markers that discriminate M. tuberculosis (Mtb) infection outcomes in an endemic setting of Ethiopia. [14] Female genital tuberculosis (TB) is an important cause of significant morbidity, short- and long-term sequelae especially in infertility in which incidence varies from 5 to 15% cases in India. [15] World Health Organization (WHO) promoted a new effective TB control based on five essential elements called the Directly Observed Treatment Short Course (DOTS) strategy. [16] Pulmonary TB (PTB) is the commonest and most infectious form of TB, but extra-PTB (EPTB) is becoming more rampant. [17, 18] Standard TB treatment includes 2 months of rifampicin, isoniazid, ethambutol and pyrazinamide followed by 4 months of rifampicin and isoniazid. Rifampicin and isoniazid both demonstrate concentration dependent killing of Mycobacterium tuberculosis. [19, 20]

History
M. tuberculosis, then known as the "tubercle bacillus", was first described on 24 March 1882 by Robert Koch, who subsequently received the Nobel Prize in Physiology or Medicine for this discovery in 1905; (21). In 1720, though, the history of tuberculosis started to take shape into what is known of it today; as the physician Benjamin Marten described in his A Theory of Consumption, [22] The M. tuberculosis complex evolved in Africa and most

probably in the Horn of Africa [23, 24]. The main human-infecting species have been classified into seven lineages. Translating these lineages into the terminology used for spoligotyping, a very crude genotyping methodology, lineage 1 contains the East African-Indian (EAI), the Manila family of strains and some Manu (Indian) strains; lineage 2 is the Beijing group; lineage 3 includes the Central Asian (CAS) strains; lineage 4 includes the Ghana and Haarlem (H/T), Latin America-Mediterranean (LAM) and X strains; types 5 and 6 correspond to M. africanum and are observed predominantly and at high frequencies in West Africa. A seventh type has been isolated from the Horn of Africa. [25].
Lineages 2, 3 and 4 all share a unique deletion event (tbD1) and thus form a monophyletic group. [26] Types 2 and 3 are more closely related to each other than to the other types. Types 5 and 6 are most closely aligned with the species that do not normally infect humans. Type 3 has been divided into two clades: CAS-Kili (found in Tanzania) and CAS-Delhi (found in India and Saudi Arabia). [27]. A much-cited study reported that M. tuberculosis has co-evolved with human populations, and that the most recent common ancestor of the M. tuberculosis complex evolved between 40,000 and 70,000 years ago [28] However, a later study that included genome sequences from M. tuberculosis complex members extracted from three 1,000-year-old Peruvian mummies, came to quite different conclusions. This study, relying on ancient DNA, estimated that the most recent common ancestor of the M. tuberculosis complex lived only 4,000 - 6,000 years ago. [29] If the most recent common ancestor of the M. tuberculosis complex were 40,000 to 70,000 years old, this would necessitate an evolutionary rate much lower than any estimates produced by genomic analyses of heterochronous samples. [30]

Significant Gap in Research

Mycobacteria are slender rods with lipid rich cell walls that are resistant to penetration by chemical dyes. These are non-motile and do not form spores. The cell wall contain Beta - hydroxylated fatty acids (Mycolic acids). The ability of Tuberculosis to grow even in immunologically activated macrophages and to remain viable with in the host for decades, are unique characteristics of tuberculosis. Isolation of the organism is essential for determining its antibiotic sensitivity in addition to confirming the specific identity of the bacillus by growth and biochemical characteristics. The drugs are effective in the pulmonary form of tuberculosis, sputum, acid fast bacteria smears become negative and the patient becomes Non-infectious in two or three weeks. [31]
Mycobacterium tuberculosis forms, dry, rough, raised, and irregular colony. It is creamy white first and become yellowish and buff colored later on. It is not emulsified easily. Egg based solid media like L J, Petragnani or American tradeau society medium have been used for primary isolation of M. tuberculosis from clinical samples and have been found more sensitive than agar based media. [32] There are marked differences in the ability of different Mycobacteria to cause lesions in various host species. Human and guinea pig are highly susceptible to M. tuberculosis infection, whereas fowl and cattle are resistant. M. tuberculosis and M. bovis are equally pathogenic to humans. The route of infection determines the pattern of lesions. The most frequent source of infection is the human who excretes, particularly from the respiratory tract, large number of tubercle bacilli. Close contact and massive exposure make transmission by droplet nuclei most likely. Susceptibility to tuberculosis is a function of the risk of clinical disease after infection has occurred. For the tuberculin negative person, the risk of acquiring tuberculin bacilli depends on exposure to sources of infectious bacilli. Principally sputum-positive patients. The risk is proportionate to the rate of active infection in population, crowding, socio economic disadvantage, and inadequacy of Medical care [33] For centuries, tuberculosis was a major killer disease, but the introduction of streptomycin in the late 1940s followed by isoniazid and in 1960s of rifampin, and ethambutol, revolutionized therapy, and tuberculosis came to be regarded as easily treatable condition. Regrettably, it is no longer, strains with increased virulence or exhibiting multidrug resistance are now common. TB is again a major threat, the WHO estimates that one-third of the world's population are currently infected with the bacillus. Poverty-stricken countries in Africa and Asia bear the brunt of the disease, partly because of an ominous synergy between M.tuberculosis and HIV. About one-third of HIV-associated deaths are caused by TB. The disease is out of control in many countries and is now the world's leading cause of death from a single agent. [34]

Major Advances and Discoveries

M.tuberculosis is resistant to multiple drugs. All Mycobacteria are acid fast. TB symptoms include fever, night sweats, weight loss and hemoptysis. Cold factor in virulent strains inhibits macrophage maturation and induces release of TNF-alpha Sulfa tides (Surphase glycolipids) inhibit phagolysosome fusion [35] although mycobacterial factors have been identified (resuscitation-promoting factor), little is known of the mechanisms of reactivation of these dormant foci. It appears that with the failure of the host to control growth of MTB the rising load of mycobacterial protein stimulates a progressively auto destructive DTH response. In addition to the TH1 response, NKT cells that recognize mycobacterial lipid antigens bound to CD1 on antigen-presenting cells, or T cells that express a γδT-cell receptor, also make IFN-γ. However, it is clear that TH1 cells have a central role in this process, since defects in any of the steps in generating a TH1 response result in absence of resistance and disease progression.
Immunity to tuberculosis is primarily related to the development of reactions mediated through CD4 T lymphocytes via TH1 pathways. Intracellular killing of MTB by macrophages activated by INF-γand other cytokines is the essential step. The specific components of MTB that are important in initiating these reactions are not known. Cytotoxic CD8 T cells are also generated during infection and may play some role. Although antibodies are formed in the course of disease, there is no evidence they play any role in immunity. [36]

Where the Research go next?

Streptomycin, an aminoglycoside antibiotic, one of the first effective agents for TB, appears to be greater Against extracellular organisms, due to streptomycin-resistant organisms may be treated with kanamycin and amikacin, to which these bacilli usually susceptible.
Pyrazinamide is a synthetic, orally effective short-course agent used in combination with isoniazid, rifampin, and ethambutol. The precise mechanism of action is unclear. Pyrazinamide must be enzymatically hydrolyzed by pyrazinamidase to pyrazinoic acid, which is the active form

of the drug. Some resistant strains lack the pyrazinamidase enzyme. Pyrazinamide is active against tuberculosis bacilli in acidic lesions and macrophages.

Ethambutol, is bacteriostatic and specific for mycobacteria. Ethambutol inhibits arabinosyl transferase-an enzyme important for the synthesis of the mycobacterial cell wall. Ethambutol is used in combination with pyrazinamide, isoniazid, and rifampin pending culture and susceptibility data. (Note: ethambutol may be discontinued if the isolate is determined to be susceptible to isoniazid, rifampin, and pyrazinamide].The risk of optic neuritis increases with higher doses and in patients with renal impairment. Visual acuity and color discrimination should be tested prior to initiating therapy and periodically thereafter. Uric acid excretion is decreased by ethambutol, and causation should be exercised in patients with gout. [37] Isoniazid is a prodrug activated by a mycobacterial catalase-peroxidase (KatG). Isoniazid targets the enzymes acyl carrier protein reductase (InhA) and β-ketoacyl-ACP synthase (KasA), which are essential for the synthesis of mycolic acid. Inhibiting mycolic acid leads to a disruption in the bacterial cell wall.

Rifampin has broader antimicrobial activity than isoniazid and can be used as part of treatment for several different bacterial infections. Because resistant strains rapidly emerge during monotherapy, it is never given as a single agent in the treatment of active tuberculosis. Rifampin blocks RNA transcription by interacting with the β-subunit of mycobacterial DNA-dependent RNA polymerase. Rifampin is generally well tolerated. The most common adverse reactions include nausea, vomiting, and rash. Hepatitis and death due to liver failure are rare. However, the drug should be used judiciously in older patients, alcoholics, or those with chronic liver disease. When rifampin is dosed intermittently, especially with doses of 1.2 g of greater, a flu-like syndrome can occur, with fever, chills, and myalgia, sometimes extending to acute renal failure, hemolytic anemia, and shock. [38]

Current Debate

Cellular components such as cell wall- induces resistance to infection, because of delayed hypersensitivity, can replace whole cell in Freund's adjuvant. Tuberculoprotein-elicits tuberculin reaction, induces delayed hypersensitivity and induces formation of Epithelioid and giant cells. Polysaccharides-induce immediate hypersensitivity and causes exudation of neutrophils from blood vessels. Lipids-causes accumulation of macrophages and neutrophils. Phospholipids-induce formation of tubercles. [39]

Candidate for treatment of LTBI are identified by TST or IGRA of persons in defined high-risk groups. For skin-testing, 5 tuberculin units of polylobate-stabilized PPD should be injected intradermal into the volar surface of the forearm (Monteux method). Multipicture tests are not recommended. Reactions are read at 48-72 H as the transverse diameter (in millimeters) of induration; the diameter of erythema is not considered. In some persons, TST reactivity wanes with time but can be recalled by a second skin test administered >1 week after the first (i.e. two-step testing). For persons periodically undergoing the TST, such as health care workers and individuals admitted to long-term care institutions, initial two-step testing may preclude subsequent misclassification of persons with boosted reactions as TST converters. The cutoff for a positive TST (and thus for treatment) is related both to the probability that the reaction represents true infection and to the likelihood that the individual, if truly infected, will develop TB. Thus, positive reactions for close contacts of infectious cases, persons with HIV infection, persons receiving drugs that suppress the immune system, and previously untreated persons whose chest radiograph is consistent with healed TB are defined as an area of induration > 5 mm in diameter. A 10-mm cutoff is used to define positive reactions in most other at-risk persons. For persons with a very low risk of developing TB if infected, a cutoff of 15 mm is used. (Except for employment purposes where longitudinal screening is anticipated, the TST is not indicated for these low-risk persons). Treatment should be considered for persons from TB-endemic countries who have a history of BCG vaccination. A positive IGRA is based on the manufacturer's recommendations. For the ELI spot assay, there is an uncertainty zone (5-7 spots) for which epidemiologic and clinical factors guide the decision to implement treatment for LTBI. This approach has also been suggested for interpretation of results in the whole-blood assay that are close to the recommended cutoff for a positive test (0.35 IU or IFN-ɣ). Some TST- and IGRA-negative individuals are also candidates for treatment. Infants and children who have come into contact with infectious cases should be treated and should have a repeat skin test 2 or 3 months after contact ends. Those whose test results remain negative should discontinue treatment. HIV infected persons who have been exposed to an infectious TB patient should receive treatment regardless of the TST result. Any HIV-infected candidate for LTBI treatment must be screened carefully to exclude active TB, which would necessitate full treatment [40]

Conclusion

Research is equally important for making appropriate changes in the strategies from time to time. And programme is coordinating with Indian Council of Medical Research (ICMR), Department of Health Research which has formed India TB Research Consortium in partnering with Department of Biotechnology (DBT), Defense Research and Development Organization (DRDO), Council of Scientific& Industrial Research (CSIR), Department of Science and Technology (DST), Department of Pharmaceuticals, World Health Organization (WHO), Bill & Melinda Gates Foundation (BMGF), Tata Trusts, The Union and the Tuberculosis Association of India. [41] The systematic review indicates that the research studies on TB among tribal population are very few. There is a need to invest and encourage researcher to work on the research plans for the control of TB in tribal areas. [42] Some species of the Mycobacterium tuberculosis complex (MTBC), particularly Mycobacterium tuberculosis, which causes human tuberculosis (TB), are the first cause of death linked to a single pathogen worldwide. In the last decades, evolutionary studies have much improved our knowledge on MTBC history and have highlighted its long co-evolution with humans. Its ability to remain latent in humans, the extraordinary proportion of asymptomatic carriers (one-third of the entire human population), the deadly epidemics and the observed increasing level of resistance to antibiotics are proof of its evolutionary success.The pollution choking most Indian cities and causing premature deaths due to pulmonary and cardiac diseases can be hazardous in yet another way. Air pollution makes a person more susceptible to tuberculosis and public health experts say that high levels of microscopic

particulate matter is probably adding to the large number of cases of TB in the country [43] From an ancient texts and historic documents, Medical historians have uncovered a wealth of information about the history of the disease. Now thease written records are being supplemented by powerful new methods for studying impact that diseases have had on people and populations in the past. One of these new methods is ancient DNA analysis, writes Professor of Molecular Archeology Terry Brown.Ancient TB is not closely related to modern human TB.The term "ancient DNA"describes the small amounts of DNA that are some-times preserved in skeletons, mummies and other dead biological remains.The public has had long standing fascination with ancient DNA, beginning 25 years ago when Jurassic park first hit the screen. [44]

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