Review of Literature
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i) **Tuberculosis Overview**

Tuberculosis (TB) remains to be one of the most pernicious of the infectious diseases, affecting one third of world’s human population caused by the tubercle bacillus Mycobacterium tuberculosis (*M. tuberculosis*) (Russell et al., 2010). *M. tuberculosis* previously known as Phthisis, Consumption, Disease of malnutrition and Great white plague has been documented in ancient civilization of Egypt more than 5000 years ago. Phthisis was identified as the most widespread disease by Hippocrates around 460BC. The disease was believed to be hereditary, and a result of the individual's mental and moral weaknesses. Clarissimus Galen, the most eminent Greek physician after Hippocrates, defined phthisis as an ulceration of the lungs, chest or throat, accompanied by coughs, low fever and wasting away of the body because of pus (Pease, 1940).

On 24th March, 1882 Robert Koch announced to the Berlin Physiological Society that he had discovered the cause of TB and made his famous presentation “Die Aetiologie der Tuberculose”. He concluded saying “…the bacilli present in tuberculous lesions do not only accompany tuberculosis, but rather cause it. These bacilli are the true agents of tuberculosis” (Kaufmann and Schaible, 2005). Three weeks later, on April 10, he published an article entitled ‘The etiology of tuberculosis’. This immense discovery, however, was not made from scratch, but involved the combining of previous scientific knowledge, chiefly the previous demonstration by the French doctor Jean-Antoine Villemin that tuberculosis was a transmissible disease, and two innovations a new staining procedure that allowed R. Koch to consistently observe the new organism in tuberculous lesions, and use of a solidified, serum-based medium instead of broths for the culture. These innovations allowed R. Koch not only to isolate *M. tuberculosis* from animal and patient specimens for the first time, but also to reproduce the disease in experimentally inoculated guinea pigs. It is thanks to Robert Koch that one of the most lethal diseases in human history could be diagnosed, could be treated and cured after the discovery of streptomycin 65 years later, and could be efficiently prevented by isolation of cases(Cambau and Drancourt, 2014).
In the wake of Koch’s discovery, subsequent progress in conquering TB has been relatively slow. Still, the ‘captain of death’, *M. tuberculosis*, today TB is the leading killer of people living with HIV. In 2012 8.6 million people fell ill with TB and total of 1.3 million people died from TB in 2012 (including 320000 people with HIV) and about 80% of reported TB cases occurred in 22 countries in 2012. The brilliant discovery of the tubercle bacillus, *M. tuberculosis* by Robert Koch, followed by the discovery of several anti-tubercular drugs in the 1940s and 1950s had made people all over the world hopeful about the eradication of TB. *M. tuberculosis* notorious success as extremely adapted pathogen was again well revealed in the World Health Organization report (WHO) 2013 which shows that. Multidrug-resistant TB (MDR-TB) does not respond to standard treatments and is difficult and costly to treat.

(a) *The tubercle bacillus*

The tubercle bacillus belongs to the genus *Mycobacterium*, the only genus of the Mycobacteriaceae family. *M. tuberculosis*, the scourge of humanity, is one of the most successful and scientifically challenging pathogen of all times. The majority of the (>50) species that comprise of the genus Mycobacterium are nonpathogenic environmental bacteria related closely to the soil bacteria Streptomyces and Actinomyces. However, a few species are highly successful pathogens, including *Mycobacterium tuberculosis*, *M. leprae*, and *M. ulcerans*, the causative agents of tuberculosis, leprosy and Buruli ulcers, respectively.

![Figure 1: Mycobacterium strains used in this study: (A) Scanning electron microscopy image of H37Rv, (B) H37Ra stained with fluorescent rhodamine, (C) H37Rv-GFP transformed with pMV261-GFP vector.](image)
Apart from *M. tuberculosis* and *M. leprae*, the genus Mycobacterium comprises several pathogens and opportunistic pathogens such as organisms belonging to the *M. avium* complex, responsible for opportunistic infections in the advanced stages of AIDS; *M. bovis*, responsible for bovine tuberculosis; *M. africanum*; *M. paratuberculosis* of the extended *M. avium* complex (MAC) family, responsible for Johne’s disease and implicated in Crohn’s disease; and *M. marinum* responsible for swimming pool granuloma. *M. tuberculosis* is mainly characterized as a fastidious, slowly growing, strictly aerobic, lipid-rich, hydrophobic, bacterial rod (2-5 μm long and 0.2-0.3 μm thick), non-motile, without capsule or spore and exhibits true branching. When stained with Ziehl-Neelsen method, these bacteria appear under microscopic examination as slightly curved or straight, small red or pink rod.

**(b) Drug-resistant tuberculosis**

TB organisms resistant to the antibiotics used in its treatment are widespread and occur in all countries surveyed. Drug resistance emerges as a result of inadequate treatment and once TB organisms acquire resistance they can spread from person to person in the same way as drug-sensitive TB (Aziz et al., 2006). Multidrug-resistant TB (MDR-TB) is caused by organisms that are resistant to at least two most effective anti-TB drugs, isoniazid and rifampicin. Extensively drug-resistant TB (XDR-TB) is a form of TB caused by organisms that are resistant to isoniazid and rifampicin (i.e. MDR-TB) as well as any fluoroquinolone and any of the second line anti-TB injectable drugs (amikacin, kanamycin or capreomycin). Rifampicin-resistant TB (RR-TB) is caused by organisms that are resistant to rifampicin, with or without resistance to other drugs. Both MDR-TB and XDR-TB are forms of RR-TB.

These forms of TB do not respond to the standard six month treatment with first line anti-TB drugs and can take two years or more to treat with drugs that are less effective, more toxic and more expensive. Globally in 2012, an estimated 450 000 people developed multidrug-resistant TB (MDR-TB) and there were an estimated 170 000 deaths from MDR-TB. The number of people diagnosed with MDR-TB nearly doubled between 2011 and 2012, and reached 94 000 worldwide. This includes 84 000 with confirmed MDR-TB and 10 000 with rifampicin resistance detected by Xpert MTB/RIF (World Health Organisation., 2013c).
(c) *HIV-associated TB*

In 2012, 1.1 million (13%) of the 8.6 million people who developed TB worldwide were HIV-positive. 75% of these HIV-positive TB cases were in the African Region. Although the number of people dying from HIV-associated TB has continued to fall globally and in most regions including the African Region, there were still 320,000 deaths from HIV-associated TB in 2012, with approximately equal numbers among men and women. UNAIDS and the Stop TB Partnership have set a target of halving TB mortality rates among people who are HIV-positive by 2015 compared with 2004 (World Health Organisation., 2013b).

(d) *Tuberculosis & diabetes*

People with a weak immune system, as a result of chronic diseases such as diabetes, are at a higher risk of progressing from latent to active TB. People with diabetes have a 2-3 times higher risk of TB compared to people without diabetes. About 10% of TB cases globally are linked to diabetes (WHO fact sheet, 2013). A large proportion of people with diabetes as well as TB is not diagnosed, or is diagnosed too late. Early detection can help improve care and control of both. People with diabetes who are diagnosed with TB have a higher risk of death during TB treatment and of TB relapse after treatment. WHO-recommended treatments should be rigorously implemented for people with TB/diabetes. Diabetes is complicated by the presence of infectious diseases, including TB. It is important that proper care for diabetes is provided to those that are suffering from TB/diabetes.

(e) *Tuberculosis in India*

The majority of TB cases worldwide in 2012 were in the South-East Asia (29%), African (27%) and Western Pacific (19%) regions. India and China alone accounted for 26% and 12% of total cases, respectively. About 75% of the estimated 2.9 million missed cases people who were either not diagnosed or diagnosed but not reported to NTPs were in 12 countries. In order of total numbers, India reports 31% of the global account. A total of 94,000 TB patients eligible for MDR-TB treatment were detected in 2012: 84,000 people with confirmed MDR-TB, plus 10,000 with rifampicin resistance detected using Xpert MTB/ RIF.
This was a 42% increase in detected cases eligible for treatment compared with 2011. The largest increases between 2011 and 2012 were in India, South Africa and Ukraine. India and South Africa accounted for about one-third of global TB deaths (Global tuberculosis Report, WHO, 2013). The rate of new TB cases has been falling worldwide for about a decade, achieving the millennium development goals (MDG) global target. TB incidences rates are also falling in all six WHO regions, while the rate of decline (2% per year) remains slow. Globally by 2012, the TB mortality rate had been reduced by 45% since 1990. The target to reduce deaths by 50% by 2015 is within reach. However, worldwide and in most countries with a high burden of MDR-TB, less than one in four of the people estimated to have MDR-TB in 2012 were detected. Just over 77 000 people with MDR-TB were started on second-line treatment in 2012, leaving at least 16 000 detected patients without treatment. At least one case of extensively drug-resistant TB (XDR-TB) has been reported by 92 countries by the end of 2012. On average, an estimated 9.6% of MDR-TB cases have XDR-TB (World Health Organisation., 2013a).

Figure 2: Global view of Tuberculosis. A) Number of MDR-TB cases estimated to occur among notified pulmonary TB cases, 2012. B) Estimated TB incidence: top-ten countries, 2012. (Adapted from Global tuberculosis Report, WHO, 2013)
ii) Treatment

Albert Schatz and Selman Waksman’s discovery of streptomycin was the first initiative towards development of an anti-TB drug. Unfortunately, the euphoria surrounding this discovery ended with bacterial resistance to the drug. Thereafter, trials proved that combination therapy prevented the drug resistance. Internationally approved drug regimens are now available for the treatment of tuberculosis. Nearly 60 years following the identification of the first antibiotic active against *M. tuberculosis*, the current recommended treatment of drug-susceptible TB is of at least 6 months duration and achieves cure rates of >95% when administered under DOT. Treatment requires a minimum of 6 months in two phases: 2 months of four drugs (isoniazid, rifampicin, pyrazinamide and ethambutol) in the intensive phase followed by 4 months of isoniazid plus rifampicin in the continuation stage (the so-called short-course chemo-therapy). This regimen is currently implemented for pulmonary TB and most forms of extrapulmonary TB regardless of HIV status (Tuberculosis Coalition for Technical Assistance, 2007).

(a) Classification of drugs in tuberculosis treatment

Anti-tuberculosis (TB) drugs are classified into five groups based on evidence of efficacy, potency, drug class and experience of use (World Health Organisation, 2010). All first-line anti-TB drug names have a standard three-letter and/or a single-letter abbreviation. In the United States rifampicin is called rifampin. First-line anti-TB drugs (Group 1) are currently recommended in a four-drug combination for the treatment of drug-susceptible TB. Second-line anti-TB drugs (Groups 2, 3 and 4) are reserved for drug-resistant TB. Third-line anti-TB drugs (Group 5) have unclear efficacy or undefined role

(i) First-line anti-TB drugs Group 1

Oral: isoniazid (H/Inh), rifampicin/rifampin (R/Rif), pyrazinamide (Z/Pza), ethambutol (E/Emb), rifapentine (P/Rpt) or rifabutin (Rfb).

(ii) Second-line anti-TB drugs Group 2

Injectable aminoglycosides: streptomycin (S/Stm), kanamycin (Km), amikacin (Amk). Injectable polypeptides: capreomycin (Cm), viomycin (Vim).
(iii) **Group 3**

Oral and injectable fluoroquinolones: ciprofloxacin (Cfx), levofloxacin (Lfx), moxifloxacin (Mfx), ofloxacin (Ofx), gatifloxacin (Gfx).

(iv) **Group 4**

Oral: para-aminosalicylic acid (Pas), cycloserine (Dcs), terizidone (Trd), ethionamide (Eto), prothionamide (Pto), thioacetazone (Thz), linezolid (Lzd).

(v) **Third-line anti-TB drugs Group 5**

Clofazimine (Cfz), linezolid (Lzd), amoxicillin plus clavulanate (Amx/Clv), imipenem plus cilastatin (Ipm/Cln), clarithromycin (Clr)

TB management is challenging, requiring accurate and early diagnosis, drug-resistance screening and the administration of effective treatment regimens for at least 6 months through directly observed therapy (DOT) and follow-up support. There is a critical need for the development and more efficient evaluation of new TB drugs and shorter treatment regimens. The hard work of researcher has culminated in historic advances in TB therapeutics, including the latest submissions to regulatory agencies for approval of two new drugs: delamanid (previously known as OPC67683) and bedaquiline (also known as TMC207 or R207910).

The WHO has suggested a treatment strategy for detection and cure of TB, "Directly Observed Treatment, Short-course" (DOTS). DOTS combine five elements: political commitment, microscopy services, drug supplies, surveillance and monitoring systems and use of highly efficacious regimes with direct observation of treatment. As per DOTS, once patients with infectious TB (bacilli visible in a sputum smear) have been identified using microscopy services, health and community workers and trained volunteers observe and record patients swallowing the full course of the correct dosage of anti-TB medicines for six to eight months (WHO Report factsheet, 2007).

TB treatment program deficiencies resulting in interrupted drug supply, non-adherence of patient to a lengthy treatment course, and co-morbidities and co-infections compromise the effectiveness of TB therapy by causing the delivery of sub-optimal drug levels. This can lead to treatment failures and the development of drug-
resistant strains of *M. tuberculosis* (acquired resistance). MDR-TB is present in virtually all countries recently surveyed by WHO and partners. MDR-TB and XDR-TB renders a definite danger and necessitates close attention to infection control that exceeds universal precautions as they are routinely and half-heartedly applied during most embalming scenarios.

**Figure 3:** Antitubercular Drugs and Their Targets: Standard “short-course” tuberculosis chemotherapy consists of 2 months of treatment with rifampicin, isoniazid, pyrazinamide, and ethambutol followed by four months on rifampicin and isoniazid. The targets of each member of the first-line quartet have been identified except for pyrazinamide, whose mechanism remains controversial. Drugs such as isoniazid, rifampicin, and the fluoroquinolones require active bacterial replication for activity, whereas pyrazinamide, nitroimidazoles, and TMC207 (Bedaquiline) may be active against dormant bacilli. (Adapted from Goldberg et al., 2012).

More concerning is the emergence of XDR-TB in people living with HIV/AIDS, where it has been associated with a more than 90% fatality rate (Stop TB Partnership and WHO 2007 Factsheet). In addition, in countries with high rates of TB, new drug-resistant cases can result from the transmission of already resistant organisms between individuals (primary resistance). This adds special urgency to the need for effective infection control and inpatient management, especially for countries with limited hospital infrastructure (Goldberg et al., 2012).
The 2011 WHO MDR-TB treatment guidelines recommend that the intensive phase of therapy is administered for at least 8 months for patients newly diagnosed with MDR-TB (Falzon et al., 2011). Regimens should include at least four second-line drugs that will have nearly certain effectiveness and be given on a daily basis under DOTs throughout the treatment duration. Total duration of therapy should be for at least 20 months when there is no history of previous MDR-TB treatment, and 28 months if there was previous MDR treatment. Pyrazinamide (Group 1) and an injectable drug are given only during the intensive phase. Durations for each phase should be modified according to the patient’s response to therapy. Group 3 contains the fluoroquinolones (discussed in more detail below), of which moxifloxacin and levofloxacin are most active. The optimal dose for each is not clearly established, although moxifloxacin is widely dosed at 400 mg daily and levofloxacin at 750 mg daily. Studies are currently planned to further refine the optimal dose of levofloxacin in MDR-TB. Other approved second-line drugs for MDR-TB treatment included in Group 4 and Group 5 have either weak or unclear bacteriostatic activity, many of which also have very high rates of side effects and intolerance. As such, drugs in these groups are generally reserved for patients with MDR-TB for whom options available in forming an adequate treatment regimen are limited. Linezolid (an oxazolidinone) and clofazimine (a riminophenazine) are two drugs in Group 5 that are undergoing additional investigation to better define their safety, tolerability and efficacy as potential repurposed drugs for MDR-TB. XDR-TB takes substantially longer to treat than MDR-TB and requires the use of third-line anti-TB drugs, which are expensive and often have more side effects than first-line or second-line drugs (Chang and Yew, 2013).

In March 2012, the WHO convened an expert consultation that identified numerous concerns about this newly proposed categorization for highly drug-resistant TB, including poor reproducibility of DST results for second-line drugs across various microbiology laboratories, even those with significant expertise (Migliori et al., 2012). In addition, worldwide, few laboratories can accurately perform DST on all second-line and third-line drugs. Labelling patients as having TDR-TB is likely to generate additional and unnecessary stigma, and as such should be avoided, particularly given the serious concerns raised by the WHO. Moreover, since new
drugs are being developed and evaluated to combat drug-resistant strains of *M. tuberculosis*, and many drugs are being repurposed, the categorization will soon become obsolete (World Health Organization, 2012).

**(b) Host intended therapies for tuberculosis treatment**

An successful host immune system is vital for the eradication of *Mycobacterium tuberculosis* infection or its containment as latent tuberculosis infection (LTBI) (Wallis et al., 2013; Zumla et al., 2013). The steady LTBI state is achieved by the ability of *M. tuberculosis* to attenuate and evade host mycobactericidal responses. Inadequate immunity leads to mycobacterial multiplication and clinical disease. Equilibrium of host and *M. tuberculosis* factors can lead to excess, but ineffective, host inflammatory responses with resultant tissue destruction. A number of options are being explored for the rational development of host aimed adjunct therapies that could reduce these destructive inflammatory responses, or augment protective immunity to enhance disease resolution, advance treatment outcomes and reduce duration of therapy.

**(i) Plummeting harsh inflammatory responses**

* a. Animal experiments

Animal experiments immunomodulation can reduce excessive inflammation and therefore restrict pathology (Subbian et al., 2011; Tobin et al., 2012b). A possible role for pro-inflammatory and anti-inflammatory eicosanoids in regulating tumour necrosis factor-α levels and in tailoring TB treatment based on host genotype has been suggested (Skerry et al., 2012; Tobin et al., 2012a). When administered prophylactically or therapeutically, the ABL family tyrosine kinase inhibitor imatinib reduced mycobacterial load and granulomatous lesions in *M. tuberculosis* infected organs and was also effective against a rifampicin-resistant strain of *M. tuberculosis* when co-administered with current first-line TB drugs (Napier et al., 2011).

* b. Repositioning drugs*

Attachment treatment for TB in experimental mouse models using generic, non-steroidal anti-inflammatory and analgesic drugs, which are widely used clinically, can alleviate the lung pathology of TB (Amaral et al., 2007b). Experimental
models of Mycobacterium marinum infected zebrafish larvae indicate that bacterial efflux pumps induce multi-drug tolerance in growing mycobacteria within macrophages. Efflux pump inhibitors such as verapamil and reserpine can reduce macrophage-induced drug tolerance (Ivanyi and Zumla, 2013; Amaral et al., 2007a), which suggests that they could be added to standard TB therapy to shorten the duration of curative treatment. The anti-nematode drug ivermectin has bactericidal properties against M. tuberculosis (Lim et al., 2013). The use of phosphodiesterase inhibitors cilostazol and sildenafil, when added to the standard TB treatment regimen, resulted in improved resolution of tissue pathology, faster mycobacterial clearance and shortened the time to lung sterilization by 1 month in comparison to the standard TB treatment regimen alone (Maiga et al., 2012).

(ii) **Adjunct immunotherapies**

The add-on immunotherapy approaches being pursued include the following: use of mycobacterial-specific antibodies; use of mycobacterial antigens or whole-cell inactivated environmental mycobacterial preparations to enhance the protective responses elicited by T-helper 1 cells; use of cytokines such as interleukin-2, interleukin-7 and interferon-γ; inhibiting prostaglandin E2 synthesis; and use of autologous bone marrow-derived stromal cell or stem cell infusions. Adding together, modulation of signal transduction pathways of the host to increase acidification of the phagosome can destroy *M. tuberculosis* bacilli1 (Bruns et al., 2012; Kuijl et al., 2007).

(c) **New anti-tuberculosis drug development**

After five decades of near stillness in TB drug development, the past 5 years has seen the emergence of a promising TB drug pipeline. Combining these new drugs with existing TB drugs offers hope for regimens that are better tolerated, shorter in duration and with fewer drug–drug interactions when compared with existing regimens. A number of new therapeutic agents are concurrently under investigation and new treatment regimens are in clinical trials. There are several drug candidates in Phase II and Phase III clinical trials together with much activity in the hit-to-lead and lead optimization stages. There is, however, a worrying gap corresponding to late preclinical development and Phase I clinical trials that needs to be addressed in order
to maintain a continuum of clinical activity and to compensate for possible attrition among the more advanced candidates.

**Figure 4:** Current global pipeline of new tuberculosis drugs: Agents currently in discovery or development for the treatment of tuberculosis (TB) are shown. Ongoing projects without a lead compound series can be viewed at the Working Group on New TB Drugs-Discovery Portfolio website. GLP, good laboratory practice; InhA, enoyl-CoA reductase; LeuRS, leucyl-tRNA synthetase. (Adapted from Zumla et al., 2013)

Many of the candidates currently in clinical trials are drugs that were developed to treat other infectious diseases (for example, fluoroquinolones, rifamycins, oxazolidinones and riminophenazines) and have since been repurposed. Several additional novel compounds are in preclinical development for TB, including the nitroimidazole TBA-354, the fluoroquinolone DC-159, the dipiperidinedine SQ609, the capuramycin SQ641, the benzothiazinone BTZ043 and the caprazene nucleoside CPZEN-45. Some of these novel compounds were back-ups from existing chemical families, but no details on their specific characteristics have been published.

(i) **Repositioning of compounds**

a. **Fluoroquinolones**

Fluoroquinolones target DNA gyrase and DNA topoisomerase in many bacteria and are frequently used for the treatment of MDR-TB as components of second-line regimens. Attention in their use as possible first-line drugs was
rehabilitated when it was shown that fluoroquinolones had the potential to reduce the duration of therapy in murine models of TB. Gatifloxacin and moxifloxacin are currently in Phase III clinical trials to establish whether drug-susceptible TB can be effectively treated in 4 months by substituting gatifloxacin for ethambutol, or moxifloxacin for ethambutol or isoniazid (Ma et al., 2010). Since fluoroquinolones are broadly available, and used to treat many infectious diseases, concern has arisen regarding the development of drug resistance in patients with undiagnosed TB who are being treated with fluoroquinolones for other conditions. To counter this, efforts are underway to identify new inhibitors that are not based on the fluoroquinolone pharmacophore as DNA gyrase is a well-validated target.

b. **Rifamycine**

Which has been the backbone of TB chemotherapy for 40 years, targets the beta subunit of RNA polymerase, thereby preventing transcription. Rifapentine, another rifamycin, acts in the same way but has a much longer half-life than rifampicin, so achieves better exposure and thus has the potential to shorten treatment duration (Rosenthal et al., 2007). A meta-analysis (Toida et al., 1992) revealed that the optimal dose for rifampicin had never been established and the HIGHRIF trial is thus testing higher doses of rifampicin. However, a major drawback of rifamycins is that they induce cytochromes P450 in the liver, which lead to drug–drug interactions with antiretroviral agents (particularly protease inhibitors) and other TB drug candidates such as bedaquiline (Andries et al., 2005). There is therefore significant interest in developing rifamycin-free regimens.

c. **Clofazimine**

A meta-analysis of studies that used the leprosy drug clofazimine repurposed for TB treatment showed that it could have a major part to play in the treatment of MDR-TB (Dey et al., 2013). An observational study evaluated the effectiveness of standardized regimens for patients with proven MDR-TB previously untreated with second-line drugs (Van et al., 2010a). Clofazimine was also found to be active in a murine model of LTBI, which suggests that it might have wider treatment applications. The optimal dose of clofazimine, duration and route of administration require further investigation.
d. Oxazolidinones

Oxazolidinones inhibits protein synthesis by binding to the 23S rRNA in the 50S ribosomal subunit of bacteria. Linezolid, a first-generation oxazolidinone, shows tuberculostatic activity in vitro and modest activity in murine models of TB (Van et al., 2010b). Early off-label trials of linezolid in combination regimens suggested that the drug was effective against MDR-TB and definitive proof for this was recently obtained in a prospective, randomized clinical trial in patients with XDR-TB. Linezolid was effective at achieving culture conversion but 82% of patients also had clinically significant adverse events, including neuropathy and myelosuppression that were thought to be linezolid-related. Patients who received 300 mg per day had fewer adverse events than those who received 600 mg per day throughout the study. Sutezolid (also known as PNU-100480; FIG. 1), a linezolid analogue that has stronger bactericidal activity in the murine model than linezolid, is currently in Phase II clinical trials (Wallis et al., 2012; Wallis et al., 2010). Combination studies have been performed in whole-blood assays and these showed that sutezolid and TMC207 or SQ109 had additive effects (Wallis et al., 2011), whereas those including PA-824 were less than additive or antagonistic.

e. Meropenem plus clavulanate combination

M. tuberculosis is obviously resistant to β-lactam antibiotics, such as meropenem, as it produces an efficient β-lactamase, BlaC, which hydrolyses them. Recently, it was gracefully confirmed that inhibition of BlaC by clavulanate could lead to M. tuberculosis becoming susceptible to Meropenem (Dauby et al., 2011c). Meropenem acts by inhibiting dd-carboxypeptidase activity, thereby perturbing peptidoglycan biosynthesis (Kumar et al., 2012b). Meropenem and clavulanate are both approved drugs and this combination has been used with some success, in conjunction with other drugs, to treat patients with MDR-TB and XDR-TB (Dauby et al., 2011b; De et al., 2013). However, although the pharmacokinetic properties of meropenem (such as a short half-life) prevent its general use for TB treatment, this work has (Dauby et al., 2011a; Kumar et al., 2012a) identified a vulnerable target that merits further exploration.
(d) **Adverse effects associated with fluoroquinolone; Gatifloxacin**

Gatifloxacin was approved by the US Food and Drug Administration (FDA) in December 1999. After receiving US approval, glucose homeostasis adverse drug events associated with use of gatifloxacin were reported in the published medical literature (Happe et al., 2004), by the Japanese manufacturer, and by Health Canada. Use of gatifloxacin was strongly associated with glucose homeostasis abnormality (Frothingham, 2005b; Frothingham, 2005a). In meta-analysis of study between April 2002 and March 2004, we identified 788 patients treated for hypoglycemia within 30 days after antibiotic therapy. As compared with macrolide antibiotics, gatifloxacin was associated with an increased risk of hypoglycemia (Park-Wyllie et al., 2006).

In a reconstituted system, gatifloxacin and temafloxacin inhibited Kir6.2DC26 channels, which function in the absence of the SUR subunit, indicating direct action of the drugs on the Kir6.2 subunits. These results suggest that stimulation of insulin secretion by inhibition of pancreatic h-cell K⁺ ATP channels underlies the hypoglycemia caused by certain fluoroquinolone in dose-dependent manner (Saraya et al., 2004). An inhibition of the cardiac rapid delayed rectifier K⁺ current (IKr) and of the ATP-sensitive K⁺ (KATP) current seems to be involved in the mechanisms of the cardiotoxic effects and the alterations in glucose homeostasis, respectively, induced by some fluoroquinolone. The different structure activity relationships point to the fact that the binding sites for fluoroquinolones on both channels are structurally different and along with that simultaneously appearing side effects, i.e. TdP cardiac arrhythmias and abnormal glucose homeostasis, are accidental (Zunkler et al., 2006).

The complexity of the range of the undesirable effect of gatifloxacin indicates that gatifloxacin may alter multiple glycemic control mechanisms and therefore educe changeable effects, depending on the dose and/or exposure time of the compound. The facilitated glucose transporter type 1 (GLUT1) protein is ubiquitously expressed in many tissues. Disturbed GLUT1 protein function by gatifloxacin weakens the systemic glycemic control and may cause dysglycemia (Ge et al., 2007). That ciprofloxacin and levofloxacin, the two widely used fluoroquinolone antibiotics, reduce GLUT1 mRNA expression, cell surface GLUT1 protein expression and glucose transport in HepG2 cells. These results further support the possibility that
GLUT1-mediated pathway is involved in fluoroquinolone-induced dysglycemia and CNS complications (Ge et al., 2009).

It is also identified in other study that fluoroquinolones in the clinically relevant concentration range are not initiators, but rather enhancers of glucose-induced insulin secretion. The block of K⁺ATP channels appears necessary but not sufficient to explain the hypoglycemic effect of fluoroquinolone (Ghaly et al., 2009). In another set of study it was confirmed gatifloxacin acutely stimulated insulin secretion while chronically inhibited insulin biosynthesis. Their results gave an inference that gatifloxacin stimulated over-secretion of glucagon-like peptide-1(GLP-1), in turn, high levels of GLP-1 and gatifloxacin synergistically impaired insulin release, worsening hyperglycemia (Yu et al., 2013).

In addition, repeated oral administration of gatifloxacin to rats at 300 mg/kg twice a day for 7 days did not change glucose tolerance. In conclusion, gatifloxacin-induced release of histamine can contribute to an increase in the serum epinephrine concentration and hyperglycemia in normal rats. In diabetic rats, lower doses of gatifloxacin can induce hyperglycemia owing to the low level of insulin secretion that they exhibit compared with normal animals (Ishiwata and Yasuhara, 2010). A fluorescence spectrometric and chemical proteomic approach study confirms that the gatifloxacin indeed binds to enolase. Role of enolase in regulation of gatifloxacin induced dysglycemic effect is discussed (Suresh et al., 2011). A differential substrate dependence of gatifloxacin action on gluconeogenesis and mitochondrial respiration combined with a decrease in pyruvate uptake by mitochondria suggest that the inhibitory action of this drug on gluconeogenesis might result from its impairment of pyruvate transport into mitochondria (Drozak et al., 2008).

Further in addition to these studies, there are some reports which correlate insulin homeostasis perturbation in pancreatic β cells. Gatifloxacin intensely stimulates insulin secretion from mouse pancreatic islets and that glibenclamide has additive effects on gatifloxacin-induced insulin secretion. They also demonstrate that chronic gatifloxacin treatment decreases islet insulin content by reduce insulin biosynthesis, which process may be associated with gatifloxacin-induced hyperglycemia. Moreover, discontinuation of gatifloxacin results in improved insulin
secretory response (Yamada et al., 2006). In addition gatifloxacin induces Ca\(^{++}\) release from ER mediated by the ryanodine receptor, and the reaction might involve in insulin secretion. Sulfonylureas induce Ca\(^{++}\) release from GPN-sensitive acidic Ca\(^{++}\) stores, but fluoroquinolones did not (Bito et al., 2013). In one of the most important study related to uproar of glucose homeostasis by gatifloxacin suggests that GFLX induces insulin oversecretion from pancreatic islet cells in the short-term, and decrease insulin productivity or increase insulin disintegration in the long-term. These results are consistent with the clinical results of GFLX finding that hypoglycemic episodes were seen after a first single administration, and most hyperglycemic episodes were seen more than 2d after the start of administration (Tomita et al., 2007). Because the risk of potentially life-threatening dysglycemia is increased during gatifloxacin therapy, these findings have important implications for clinical practice.

**iii) Immunology of M. tuberculosis Pathogenesis**

It has long been accepted that *M. tuberculosis* invades the host in small infectious droplets in the lower respiratory tract, rather than in the upper respiratory tract.

**(a) Stage 1: Innate Immune Responses**

*M. tuberculosis* is transmitted by aerosol, and largely, if not exclusively, inhabits professional phagocytic cells in the lungs, including macrophages, neutrophils, monocytes and dendritic cells (DCs) (Kang et al., 2011; Wallgren, 1948; Wolf, 2007). As these cells are recruited, they become infected by the expanding population of mycobacteria and establish early granulomas.

The recruitment of phagocytes to sites of mycobacterial infection actually benefits the pathogen during the early stages of infection, by providing additional cellular niches for bacterial population expansion (Davis and Ramakrishnan, 2009). *Mycobacterium marinum*, have evolved multiple mechanisms to manipulate their cellular niches for their own advantage.
Mycobacteria modulate the trafficking and maturation of the phagosomes in which they reside (Chackerian et al., 2002; Khader, 2006; Blomgran et al., 2012), allowing them to evade lysosomal mechanisms of restriction, killing and degradation. Mycobacteria use several virulence mechanisms to optimize their spread from cell to cell, e.g., the ESX1 type VII secretion system, the absence of which attenuates the strain of M. bovis used in the BCG vaccine promotes the necrotic death of infected cells and the recruitment of macrophages.

M. tuberculosis possesses multiple mechanisms for inhibiting host cell apoptosis (Pym et al., 2002; Hinchey, 2007; Miller et al., 2010; Velmurugan, 2007). The expanding bacterial population spreads from cell to cell and increases the range of cell subsets that it infects to include DCs, which can subsequently initiate adaptive immune responses (Poulsen, 1950).
Figure 6: The stages in the immunological life cycle of tuberculosis. The framework for the life cycle is based on clinical, epidemiological and immunological studies in humans. Included are examples of some of the immunological mechanisms and functions that characterize each stage, in cases where they are known. Examples of mechanisms with question marks are hypothetical and are discussed in the text. Shown in the centre are examples of the known or experimentally supported states of the bacteria at distinct stages of the immunological life cycle. PAMP, pathogen- associated molecular pattern; TB, tuberculosis; TNF, tumour necrosis factor. (Adapted from Joel D. Ernst, 2012)

(i) Mechanisms of innate immunity in Tuberculosis

The innate immune stage is characterized by the recognition of M. tuberculosis components by multiple pattern-recognition receptors. Of the Toll-like receptors (TLRs), TLR2 has the largest number of identified mycobacterial agonists, including lipoproteins, phosphatidylinositol mannans and lipomannan1 (Divangahi et al., 2010; Banaiee et al., 2006). In addition, TLR9 senses mycobacterial DNA and contributes to the production of cytokines by macrophages and DCs in M. tuberculosis-infected mice (Bafica, 2005). Although deletion of Tlr2 and Tlr9, singly or in combination, does not have a marked effect on the control of M. tuberculosis in mice, deletion of the gene encoding the shared TLR adaptor
molecule MYD88 results in a rapidly lethal infection (Holscher, 2008). This is probably due to defective signalling in response to interleukin-1α (IL-1α) and IL-1β, as such signalling also depends on MYD88 (Jayaraman et al., 2013; Mayer-Barber, 2011). Additional recognition of M. tuberculosis is mediated. Additional recognition of M. tuberculosis is mediated by specific members of the C-type lectin receptor (CLR) family, including DC-SIGN (Tailleux, 2003; Tanne, 2009), dectin (Marakalala, 2011; Rothfuchs, 2007), the mincle (Ishikawa, 2009; Schoenen, 2010). Deletion of any one mannose receptor (Court, 2010; Schlesinger, 1993) and mincle (Court, 2010; Schlesinger, 1993; Schoenen, 2010). of these CLR genes has little or no effect on the course of infection, whereas deletion of the gene encoding the shared CLR adaptor molecule CARD9 is associated with accelerated mortality and excessive neutrophilic lung inflammation (Dorhoi, 2010; Nandi and Behar, 2011) of the cytosolic pattern-recognition receptors, nucleotide-binding oligomerization domain protein 2 (NOD2) (Brooks, 2011; Coulombe, 2009; Divangahi, 2008; Mishra, 2010) and NOD, LRR and pyrin domain containing 3 (NLRP3) recognize the peptidoglycan subunit N-glycolyl muramyl dipeptide and one or more ESX1-secreted substrates (such as ESAT6), respectively. Therefore, stimulation of these pattern-recognition receptors, individually or collectively, induces the expression of pro-inflammatory cytokines, selected chemokines and cellular adhesion receptors that contribute to local and systemic immune cell mobilization and activation (Cooper et al., 2011b). However, they also provide the basis for the subsequent initiation of cellular adaptive immune responses by driving the recruitment and maturation of DCs (Cooper, 2009a). Accelerating the availability of antigen-specific CD4+ effector T cells through the adoptive transfer of these cells has no effect on the survival or growth of M. tuberculosis during the first 7 days of infection (Cooper, 2009a).

(b) Stage 2: Immunological balance

(i) Deferred initiation of adaptive immunity

Measurable adaptive immune responses emerge in humans approximately 42 days after M. tuberculosis exposure and infection (Wallgren, 1948; Poulsen, 1950). In mice, the activation of M. tuberculosis antigen-specific CD4+ T cells occurs earliest in lymph nodes that drain the lungs (Chackerian et al., 2002; Reiley, 2008; Wolf,
and requires the transport of live bacteria from the lungs to the draining lymph nodes by myeloid DCs (Khader, 2006; Wolf, 2008). It is currently unclear why this step is so prolonged, although there is evidence that *M. tuberculosis* infection of myeloid DCs inhibits their migration in response to ligands for CC-chemokine receptor 7 (CCR7) (Blomgran and Ernst, 2011). In addition, the inhibition of neutrophil apoptosis by *M. tuberculosis* contributes to the delayed kinetics of adaptive immune response induction. Once bacteria are transported to the draining lymph nodes and produce antigens for presentation to naive CD4+ T cells, the proliferation, differentiation and trafficking to the lungs of effector CD4+ T cells occurs with kinetics similar to those observed with soluble protein antigens. However, *M. tuberculosis* antigen-specific regulatory T cells also develop during the course of infection and contribute to the delayed priming of CD4+ and CD8+ T cells in the lung-draining lymph nodes (Wolf, 2008).

(ii) *Arrest but not execution of bacteria*

The onset of adaptive immune responses in TB results in the arrest of the progressive growth of the bacterial population and may result in transient disease symptoms, including fever and an unusual skin rash termed erythema nodosum (Poulsen, 1950). After the onset of adaptive immunity, most humans become asymptomatic, do not shed bacteria and are considered to have latent TB infection. It is important to note that the size of the bacterial burden in human latent TB infection is unknown, owing to the current lack of available methods to determine it. Multiple mechanisms probably contribute to the limited ability of adaptive immune responses to kill *M. tuberculosis*. Such mechanisms include: impaired MHC class II-mediated antigen presentation (Noss, 2001; Pancholi et al., 1993); induction of the anti-inflammatory mediator lipoxin A4 (Divangahi et al., 2010); restriction by regulatory T cells (Scott-Browne, 2007); down regulation of bacterial antigen gene expression and, therefore, failure to induce antigen-specific CD4+ T cells; and resistance to the macrophage-activating effects of interferon-γ (IFNγ) (Fortune, 2004; Kincaid and Ernst, 2003; Ting et al., 1999). During latency a subpopulation of
bacteria continues to replicate during this chronic, clinically silent stage of infection in mice (Gill, 2009) and it also accumulates mutations during latency (Ford, 2011).

(iii) **Immunological mechanisms that contribute to equilibrium**

In addition to responses by classical MHC class I- or class II-restricted αβ T cells that recognize bacterial peptide epitopes, responses by other T cell subsets are observed. Such cells include CD1-restricted, mycobacterial lipid-specific T cells (which are predominantly CD4+)(Kasmar, 2011; Montamat-Sicotte, 2011; Heinzl, 2002; Joosten, 2010), HLA-E-restricted CD8+ T cells and mucosa-associated innate-like T cells (Gold, 2010). Although these other T cell subsets are under active investigation, their roles in immunity to TB have not yet been determined. Among the mediators of immunity to *M. tuberculosis*, tumour necrosis factor (TNF) and IFNγ are the best described in humans (Harris and Keane, 2010; Jouanguy, 2000; Jouanguy, 1997). Additional molecules that contribute to the immune control of M. tuberculosis in mice, but that have not yet been shown to be significant in humans, include IL-17, cytolytic T cell-expressed perforin and the IFNγ-induced molecules nitric oxide synthase 2 (NOS2) and LRG47 (also known as IRGM1). Further, Granulysin is a cytolytic T cell granule protein that has direct anti-mycobacterial activity in vitro (Stegelmann, 2005), although its role in controlling *M. tuberculosis* in vivo remains unknown. The vitamin D is an essential cofactor for the IFNγ-mediated induction of the anti- mycobacterial peptide cathelicidin (Fabri, 2011). Furthermore, vitamin D levels in humans are closely associated with susceptibility to active TB (Martineau, 2011). Second, it seems increasingly likely that no single parameter will mediate or correlate with protective immunity in tuberculosis, implying that increasing use of systems biology, bioinformatics and biostatistics will be needed to formulate optimal models and test them in expanded studies.

(iv) **The bacterial contribution to equilibrium**

Strong evidence exists that the mycobacteria are also active contributors to the immunological equilibrium state in latent TB. First, a well-characterized bacterial regulon that is controlled by DosR–DosS a two-component signal transduction system
in mycobacteria is induced by several stimuli thought to prevail during latent TB, including local hypoxia (Park, 2003; Sherman, 2001), nitric oxide and carbon monoxide (Voskuil, 2003; Kumar, 2008; Shiloh et al., 2008). In addition, \textit{M. tuberculosis} encodes five proteins that resemble the well-characterized \textit{Micrococcus luteus} resuscitation-promoting factor (Rpf), which is a secreted protein that has the ability to ‘resuscitate’ bacteria from a nutrient-starved dormant state. Finally, \textit{M. tuberculosis} encodes 88 toxin–antitoxin gene pairs, the expression balance of which regulates multiple phenomena, including whether the bacteria replicate or remain static (Russell-Goldman et al., 2008; Tufariello, 2006; Ramage et al., 2009). Thus, \textit{M. tuberculosis} possesses at least three systems (the dormancy regulon, resuscitation promoting factors and toxin–antitoxin gene pairs) that regulate its metabolic and growth state. Further investigation is likely to provide insights into the host and bacterial mechanisms that regulate these systems and that determine whether the bacteria remain in an equilibrium state with the host or resume growth and reactivate to cause active TB disease.

\textbf{(c) Stage 3: Reactivation of Tuberculosis}

Reactivation of latent TB reflects progression to active, symptomatic disease, which is usually characterized by the shedding of \textit{M. tuberculosis} in respiratory secretions, especially during coughing. Reactivation TB is widely attributed to ‘weakened’ immunity, although only minorities of cases are attributable to well-characterized defects in immunity.

\textbf{(i) Recognised mechanisms underlying TB reactivation}

In humans, only two mechanisms have been identified that explain reactivation TB. The first mechanism involves the quantitative and qualitative CD4+ T cell defects that occur in people infected with HIV (Kwan and Ernst, 2011; Naranbhai et al., 2014). In addition to the extensive depletion of CD4+ T cells, there is strong experimental evidence from human studies to suggest that, before this profound CD4+ T cell depletion, HIV targets and depletes \textit{M. tuberculosis} antigen-specific CD4+ T cells at a greater frequency than CD4+ T cells specific for other antigens (Geldmacher, 2010; Geldmacher, 2008). The second well-characterized mechanism that is clearly associated with reactivation TB is the therapeutic
neutralization of TNF (Harris and Keane, 2010), especially by monoclonal antibodies that is justified decreased macrophage-mediated anti-mycobacterial activity and the subsequent death of macrophages (Jung et al., 2013; Clay et al., 2008); the induction of a higher frequency of regulatory T cells (Nadkarni et al., 2007); and the depletion of a subset of CD45RA+ effector memory CD8+T cells that contain granulysin and have been shown to contribute to M. tuberculosis killing in vitro (Bruns, 2009). So finally CD4+ T cells and TNF as two of the major elements that mediate protective immunity in TB and that prevent reactivation.

(ii) **Recognised associations with other medical conditions**

These conditions include diabetes mellitus the increasing prevalence of which in developing countries is leading to the convergence of its geographical distribution with that of TB to increase the severity of the TB epidemic (Harries, 2011). Treatment with glucocorticoids is also a well-known risk factor for reactivation TB (Vallerskog et al., 2010; Jick et al., 2006). Leptin also modulates the development and function of T helper 1 (TH1) cells (Faggioni et al., 2001), suggesting a mechanism for the enhanced susceptibility to TB in thin people. Indeed, leptin-unresponsive mice poorly control M. tuberculosis infection (Lemos et al., 2011). Possible mechanism: T cell exhaustion. These data indicate that pathways that operate in exhausted CD8+ T cells in chronic viral infections have different functions in CD4+ T cells in TB. In addition, these data suggest that a complex pathogen containing multiple antigens, such as M. tuberculosis may use mechanisms other than T cell exhaustion to prevent its elimination.

(iii) **Possible method: altered antigen expression**

*M. tuberculosis* and other bacteria and parasites respond to signals from their environment to regulate their gene expression. In addition to allowing bacterial survival and growth under diverse conditions, this ability to regulate gene expression contributes to the alteration of antigen gene expression profiles at distinct stages of infection, allowing the bacteria to evade recognition by T cells specific for certain antigens. In particular, the expression of at least two antigens that are immunodominant in humans and mice ESAT6 and Ag85B is downregulated after the appearance of CD4+ and CD8+T cells in the lungs of infected mice (Faggioni et al.,
The magnitude of the reduction in gene expression is more marked for Ag85B (and the closely related antigen Ag85A) than for ESAT6 (Reiley, 2010; Barber et al., 2011; Rogerson, 2006; Shi et al., 2004; Aagaard, 2011), and the expression of genes encoding other antigens (such as HspX and Rv2660c) is maintained or increased during chronic infection. This indicates that, although the profile of antigen expression may change during infection, a distinct repertoire of antigens and T cells may contribute to the maintenance of host–pathogen equilibrium during latency.

(iv) Potential mechanism: altered cell trafficking

If cell trafficking to granulomas needs to be maintained for decades to maintain local immunity in latent TB, it stands to reason that defective cell trafficking, even if slight or intermittent, could allow for TB reactivation. In mice, transgenic overexpression of CC-chemokine ligand 2 (CCL2; also known as MCP1) (Rutledge, 1995) or the absence of CCR2 (Peters, 2001; Scott and Flynn, 2002) decreases the recruitment of monocytes and DCs to the site of M. tuberculosis infection and is associated with poorer immune control of infection. By contrast, CXC-chemokine receptor 3 (CXCR3) deficient mice are more resistant to infection and can control chronic M. tuberculosis infection in the lungs more effectively than wild-type mice (Chakravarty, 2007). In humans, several polymorphisms in genes encoding chemokines and chemokine receptors such as functional variants of CCL2, CCL3L1 and CCR5 have been associated with active M. tuberculosis infection (Flores-Villanueva, 2005; Mamtani, 2011).

(v) Can the bacteria be the primary drivers of reactivation?

In turn, M. tuberculosis also has specific programs for recovering from dormancy, suggesting that the bacteria may assume a primary role in some cases of reactivation TB that are not explained by immune defects or deficiencies.

(vi) Impulsive deactivation

Inactive TB differs from latent TB in that in the former there are often abnormalities detected on chest X-rays, whereas such findings are absent in latent TB. A recent study of long-term survivors of untreated TB revealed that approximately
70% of these individuals had CD4+ effector memory T cell responses to *M. tuberculosis* antigens, suggesting that they were persistently infected. By contrast, a substantial fraction of the remaining individuals had CD4+ central memory T cell responses, consistent with clearance of infection (Millington et al., 2010). As one especially promising example, transcriptional profiling of peripheral blood cells from humans with latent and active TB has revealed the previously unsuspected association of active TB with a type I IFN signature and with the expression of neutrophil-specific genes (Berry, 2010). It is likely that additional prospective analyses particularly of people recently exposed to active TB cases and presumably newly infected (Maertzdorf, 2011) will clarify the roles of the type I IFN and neutrophil signatures in the pathogenesis of and immunity to TB.

**(d) Stage 4: Transmission**

A require step in all infectious diseases is transmission to new hosts. In the case of TB, this occurs through the airborne route, in which bacteria are expelled (usually by coughing) from an individual with active disease and then inhaled by vulnerable hosts. As in particular, individuals with a form of TB termed cavitary TB are especially infectious (Rodrigo, 1997) Cavitary TB is the consequence of lung tissue destruction and the formation of macroscopic open spaces that contain numerous *M. tuberculosis* bacilli (Kaplan, 2003) and connect to large airways, which facilitates efficient expectoration of the bacteria.

**(i) Proof that immune responses promote transmission**

Recent systematic review revealed a linear correlation between the number of circulating CD4+ cells and the frequency of cavitary TB (Kwan and Ernst, 2011). It is unclear whether the effect of CD4+ T cells on the promotion of cavitary TB is direct or indirect, and the mechanisms by which CD4+ T cells contribute to lung tissue damage and cavitary TB are not well characterized. Although the collagen-degrading metalloproteinase MMP1 has been implicated as a mediator (Elkington, 2011), its relationship to the contributions of CD4+ T cells has not yet been established. T cell
responses probably mediate this effect by contributing to inflammatory tissue damage and lung cavitation, which promotes the transmission of the bacteria to new hosts.

Although the results in humans demonstrate an association between CD4+ T cells, cavitary TB and TB transmission, the discovery of the underlying direct and indirect mechanisms is likely to require studies in a non-human animal model.

iv) Dendritic cell in Tuberculosis

“On 3 October 2011, immunologists in many parts of the world heard that Ralph Steinman shared the 2011 Nobel Prize in physiology or medicine and fired off congratulatory emails. Hours later, they were shocked to learn of his death three days earlier from pancreatic cancer. Following an unprecedented second meeting on the day of its decision, the Nobel Prize Committee for Physiology or Medicine judiciously tempered the application of its rule against posthumous awards…”

Carl Nathan...

After thirty-five years of their discovery, DCs have now came out as key modulators of immune processes, including antimicrobial immunity, are maintained by a heterogeneous population of DCs with specialized functions. It is critical to understand the mechanisms that regulate the homeostasis of DC subsets to better use their therapeutic potential. This will require investigating the mechanisms that regulate DC development, trafficking, localization, and turnover in specialized niches in vivo. A main challenge for the future is to translate what we have learned from the mouse into humans and better explore the diversity of human lymphoid and nonlymphoid organ DC populations.

Recent advances have clarified the origin of DCs, a hematopoietic lineage specialized to present antigens and both initiate and control immunity (Heath and Carbone, 2009; Melief, 2008). In the bone marrow, a common monocyte-DC precursor (Fogg et al., 2006) gives rise to monocytes and other precursors termed common DC precursors (Naik et al., 2007) and pre-cDCs (Liu et al., 2009).

Nevertheless, monocytes also can differentiate into DCs. Although first studied as macrophage precursors, mainly in vitro (de Villiers et al., 1994a; de Villiers et al., 1994b; Johnson, Jr. et al., 1977) monocytes were later recognized to have an added potential to develop into DCs (monocyte-derived DCs [Mo-DCs]). This too has
been studied primarily in cultures of human blood monocytes (Sallusto and Lanzavecchia, 1994).

Figure 7: Differentiation of the macrophage/DC progenitor and origin of macrophage and DC subsets.
(Adapted from O’Garra et al., 2013)

Monocytes, upon culture for several days in GM-CSF and IL-4, acquire a typical probing or dendritic morphology, lose the capacity to phagocytose, and adhere to various tissue culture surfaces but acquire strong capacities to initiate immunity.

Mo-DCs can immunize humans (Dhodapkar et al., 1999; Schuler-Thurner et al., 2000) and home to the T cell areas of lymph nodes (LNs) (de, I et al., 2003). Monocytes are about 20 times more abundant than DCs in blood and marrow, so the mobilization of this monocyte reservoir in vivo to generate potent antigen-presenting DCs needs to be elucidated. DC-SIGN is a hallmark of human Mo-DCs in culture (Geijtenbeek et al., 2000b) but is not detected on the rich network of presumably monocyte-independent DCs in human LNs in the steady state (Granelli-Piperno et al., 2005). When Mo-DCs are compared functionally to classical DCs from the same LNs, the former are not only active but can be superior in stimulating the mixed leukocyte reaction (MLR) and in presenting protein antigens, administered in vitro and also in vivo prior to testing as presenting cells.
DC-SIGN/CD209 can play pathogenic roles, either in transmitting infectious agents like HIV and CMV in the case of cultured human Mo-DCs (Geijtenbeek et al., 2000a; Halary et al., 2002) or in transducing inhibitory signals as seen when human DC-SIGN/CD209 interacts with mycobacteria (Geijtenbeek et al., 2003; Tailleux et al., 2003) also have protective functions for capture and presentation of glycan-modified antigens (Tacken et al., 2005). In a LPS induced DCs differentiation study, blood monocytes drop to 20% of their normal levels 6–12 hr after i.v. LPS, and at the same time, cells move into LNs and differentiate into DC-SIGN/CD209a⁺ MMR/CD206⁺ Mo-DCs. This influx requires CCR7 and CD62L, both expressed by bone marrow and blood monocytes. Among the agonists for Toll-like receptors, only LPS via TLR4 had this capacity to induce Mo-DCs. A key feature of the Mo-DCs that are mobilized by LPS is that they express CD14, which not only proved to be an independent marker for Mo-DCs but was also essential for their generation (Cheong et al., 2010).

v) Cytokines in adaptive immunity to M. tuberculosis

Protective immune responses against M. tuberculosis are largely mediated by CD4⁺ Th1 cells, which secrete IFN-γ. Antigen-specific CD8⁺ T cells, natural killer (NK) cells, γδTcells, and CD1-restricted T cells also produce IFN-γ during M. tuberculosis infection, but, they cannot compensate for a lack of CD4⁺ T cells (Cooper, 2009b; Flynn and Chan, 2001; North and Jung, 2004). Mice are unable to control a low-dose M. tuberculosis infection in the absence of IFN-γ (Cooper et al., 2002c; Cooper et al., 2011a). They fail to produce reactive nitrogen and oxygen intermediates, and they develop progressive tissue destruction, which is associated with uncontrolled bacterial replication.

The induction of protective IFN-γ T cell responses against primary M. tuberculosis infection is dependent on IL-12 (p40/p35), which is mainly secreted by M. tuberculosis-activated DCs (Cooper, 2009b), in part via TLR-dependent mechanisms. Mice lacking IL-12p40 cannot control the growth of the bacterial infection (Cooper et al., 1997; Cooper, 2009b). It is not only IL-12 which is essential for the initial activation of IFN-γ T cell responses to M. tuberculosis, but continued IL-12p70 production is also required for the expanded and sustained IFN-γ Th1
responses in the lungs that are required to maintain control of chronic infection. Further studies have revealed different contributions of the p40 and p35 subunits of IL-12 to the control of mycobacterial infection (Cooper et al., 2002e).

![Figure 8: Regulation of the immune response during M. tuberculosis infection. Following infection with M. tuberculosis, specific regulatory pathways that normally serve to limit host-induced immune pathology may inadvertently promote pathogen persistence. Two such regulators include IL-10 and regulatory T cells. The induction of IL-10 during infection can lead to the inhibition of macrophage effector functions, with reduced bacterial killing and impaired secretion of cytokines/chemokines. IL-10 can also block chemotactic factors that control DC trafficking to the draining lymph nodes. In the lymph nodes, both IL-10 and regulatory T cells can block the differentiation of naive T cells to IFN-\(\gamma\)-producing Th1 cells, predominantly through direct effects on the DC. Furthermore, IL-10 can block T cell chemotactic factors such as CXCL10, which mediates Th1 cell trafficking back to the lungs, in addition to blocking macrophage activation and downstream antimicrobial pathways in response to IFN-\(\gamma\) (Adopted from O'Garra et al., 2013).

Mice lacking the p40 subunit were more susceptible to \textit{M. tuberculosis} infection than were p35-deficient (IL-12a\(^{-/-}\)) mice and showed increased bacterial growth, increased mortality, and reduced IFN-\(\gamma\) T cell responses compared with the
p35−/− mice (Cooper et al., 2002a). This finding suggested that IL-12p40 itself may play a protective role. IL-12p40 was found to be required for DC migration and T cell priming during M. tuberculosis infection (Khader et al., 2006a; O'Garra et al., 2013).

An alternative explanation for the greater susceptibility of p40−/− than p35−/− mice to M. tuberculosis infection is that IL-23p19 contributes to protective immunity. IL-23p19 binds with IL-12p40 to form functional IL-23, and IL-23p19 is expressed early during M. tuberculosis infection (Khader et al., 2005a; Wozniak et al., 2006).

However, IL-23p19−/− mice effectively controlled M. tuberculosis infection, and there was no reduction in IFN-γ-specific T cells or IFN-γ mRNA at the site of infection. Conversely, there was a marked reduction in IL-17-producing antigen-specific CD4+ T cells and IL-17 mRNA expression in the lungs. As IL-23p19 is not required to control M. tuberculosis infection, it is not clear whether Th17 responses play a significant protective role. In the absence of IL-12p70, IL-23 could compensate for the generation of IFN-γ-producing cells during M. tuberculosis infection (Cooper et al., 2002b; Khader et al., 2005b), although this compensatory response was insufficient to control the infection (Cooper et al., 2002d; Khader et al., 2006b). Therefore, although IL-23 can partially compensate for IL-12p70 deficiency to stimulate a Th1 response, this cytokine is not essential to control mycobacterial infection.

Mutations in the IL-12/IFN-γ axis increase susceptibility to tuberculosis. IFN-γ is the cytokine most invariably detected as protein or mRNA at the sites of human M. tuberculosis infection [including in the lung, bronchoalveolar lavage (BAL) fluid, TB pleuritis fluid, and lymph nodes] and in the responses of PBMCs to mycobacterial antigens. IFN-γ-mediated killing of M. tuberculosis infection in macrophages and control of inflammation and CD4+ T cells is necessary to inhibit host damage during M. tuberculosis infection.

During mycobacterial infections, IL-17 can also be produced by γδ T cells and a non-CD4+ CD8+ population (Lockhart et al., 2006). IL-17 may play a role in granuloma formation and Th1 enhancement following BCG infection and in granuloma formation during intratracheal infection with M. tuberculosis (Okamoto et al., 2010). However, IL-23, which is essential for the IL-17 response during TB, is
dispensable for protection and antigen-specific IFN-\(\gamma\) responses if IL-12p70 is available (Khader et al., 2005c).

IL-10 is an immunosuppressive cytokine essential for dampening the immune response and limiting host immune pathology to numerous intracellular pathogens and gut flora, but, if overproduced, IL-10 can contribute to chronic infection. IL-10 is made by many immune cells including macrophages, neutrophils, DCs, B cells, and T cells (Saraiva and O'Garra, 2010). A major mechanism whereby IL-10 achieves its effects is by inhibiting the antigen-presenting cell function of macrophages and DCs and the production of cytokines such as IL-12, thus inhibiting the development of Th1 responses (Fiorentino et al., 1991b; Fiorentino et al., 1991a; Moore et al., 2001). In addition, IL-10 can inhibit the killing of intracellular pathogens by macrophages, induction of nitric oxide, and production of TNF (Bogdan et al., 1991; Moore et al., 2001). M. tuberculosis infection of human macrophages induced the production of IL-10 (Shaw et al., 2000) and resulted in the blockade of phagosome maturation via a STAT3-dependent mechanism, resulting in M. tuberculosis survival and outgrowth. Thus, whereas IL-12p40 promotes DC migration during mycobacterial infection (Khader et al., 2006c), IL-10 may limit it to facilitate M. tuberculosis survival.

Because Th1 responses are protective against M. tuberculosis infection and Th2 responses cross-regulate and inhibit Th1 responses, we should not be surprised by reports that chronic worm infection of mice reduces immunogenicity (Elias et al., 2008) or by reports of reduced Th1 responses in active (Resende et al., 2007) and latent (Babu et al., 2009) TB patients coinfected with helminthes. It is becoming apparent that type I IFN increases susceptibility to M. tuberculosis infection via several mechanisms. The investigation of hypervirulent M. tuberculosis strains points to suppression of proinflammatory cytokines and Th1 immunity as playing a role (Stanley et al., 2007b; Ordway et al., 2007; Manca et al., 2005). The selective induction of type I IFN–associated genes and IFN-\(\beta\) occurs in macrophages infected with virulent but not a virulent M. tuberculosis with an inactive ESX-1 secretion system (Stanley et al., 2007a).
vi) MicroRNAs as Regulator of Dendritic Cell Differentiation and Function

MicroRNAs (miRNAs) are an evolutionarily ancient class of endogenous small noncoding RNAs. miRNAs transcribed in the nucleus and after processing exported into the cytoplasm. miRNAs post-transcriptionally regulate gene expression by binding to target mRNAs and initiating either their cleavage or a reduction in the translational efficiency. Since the discovery of miRNAs less than two decades ago, hundreds have now been identified in mammals, and many of them are conserved across species. miRNAs are essential for development (Bernstein et al., 2003). Multiple examples of miRNA dysregulation leading to oncogenesis implicate miRNAs in the maintenance of homeostasis (Esquela-Kerscher and Slack, 2006).

The generation of miRNAs proceeds via a specialized pathway involving the RNase Dicer that produces RNA duplexes of $\sim21$ bp in length, the mature miRNA (Krol et al., 2010). miRNA binding to target mRNAs silences gene expression by mRNA degradation or translational repression (Bartel, 2009; Fabian et al., 2010). Gene expression regulation achieved by miRNAs is complex 60% of all human protein coding genes are predicted to contain miRNA binding sites in their 3’ untranslated region (3’UTR) (Fabian et al., 2010). Expression profiling has shown that distinct cell types express unique miRNA profiles, with changing patterns during cellular differentiation and malignant transformation (Landgraf et al., 2007). The degree of silencing achieved by an individual miRNA is relatively modest, leading to the idea that miRNAs finetune gene expression (Fabian et al., 2010; Lodish et al., 2008). However, each miRNA may control the expression of hundreds of target genes, and mRNAs may have multiple miRNA binding sites, such that different miRNAs may regulate target genes coordinately or synergistically (Krek et al., 2005). Furthermore, some of the primary gene targets of miRNAs are transcription factors, rendering miRNAs regulators of the regulators, or “micromanagers” (Fabian et al., 2010). Importantly, in contrast to regulation by transcription factors, miRNAs are especially capable of inducing rapid changes in gene expression, which is of particular relevance during cellular responses to environmental cues.

More than 100 miRNAs are selectively expressed in cells of the adaptive and innate immune systems (O’Connell et al., 2010), and miRNAs are known to modulate
hematopoietic lineage commitment (Cheng et al., 2010). Multiple transgenic mouse strains have been made in which Dicer, an enzyme required for miRNA production, is specifically deleted in various cell types, thereby allowing analysis of the effect of loss of functional miRNAs. In the absence of Dicer, defective homeostasis and function have been observed in various T cell subsets (Zhou et al., 2010a), NK cells, and B cells (Koralov et al., 2008). Functions of specific miRNAs in immune cells have also been documented. For example, loss of miR-155 leads to impaired responses to pathogens or immunization due to inhibited germinal center responses and altered Th cell subset polarization (Rodriguez et al., 2007; Vigorito et al., 2007), whereas miR-181a regulates TCR signal transduction sensitivity (Ebert et al., 2009; Li et al., 2007). Several miRNAs have been implicated in innate inflammatory responses, including miR-146a, miR-132, and miR-155, which are upregulated in monocytes in response to TLR activation.

Some miRNAs are active in multiple immune cells, whereas for other miRNAs functions have been described in DCs only. This is seen via the actions of let-7i, miR-142-3p, miR-146a, the miR-148 family, miR-155 (Lu et al., 2011a; Zhou et al., 2010b), and miR-155* in regulating cytokine production in response to DC activation, and as an inherent characteristic of LCs via constitutive Mir-146a (Jurkin et al., 2010) expression. This is also reported that miRNAs regulate checkpoints of DC differentiation: miR-21 and miR-34a (Hashimi et al., 2009) are necessary for DC differentiation from monocytes, and miR-221 and miR-222 control pDC and cDC cell fates (Lu et al., 2011b). It is not only interesting to identify those miRNAs that function in DC biology, but also to consider how the rapid regulation of gene expression that is achieved by these molecules in DCs impacts on adaptive immune responses.

The finding that miRNA expression in DCs is associated with both proinflammatory and anti-inflammatory responses implies that multiple miRNAs may act in a tightly balanced tandem to regulate the signals transmitted by DCs to other cells of the immune system. The cooperative behaviour of miR-155 and miR-155* in pDCs also shows that sequential miRNA expression may influence both DC activation and subsequent contraction of immune responses (Zhou et al., 2010c),

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suggesting that further analysis of the temporal regulation of DC relevant miRNAs is warranted. DCs help to orchestrate pathogen-specific immune responses by secreting appropriate cytokines and influencing CD4+ T cell subset differentiation.

Figure 9: miRNA involvement in DC differentiation and function. A, miRNAs with functions during DC differentiation from hematopoetic precursors, in the steady state (immature DCs) and upon activation (mature DCs), from in vivo and in vitro generated DC subsets, are illustrated. Gene targets of the miRNAs are described in the text and Table I. B, The altered expression of some miRNAs following DC maturation leads to changes in DC phenotype and function that can be classified as either pro- or anti-inflammatory. The corresponding effects of altered DC function on T cell activity are indicated. Upregulated (∨) miRNAs are indicated in red, and downregulated (↓) miRNAs in green. Teff, T effector cell; Treg, regulatory T cell; costim., costimulatory molecules (Reproduced with permission from Turner M.L. et al., 2011).

In brief many miRNAs regulate DC cytokine production. The finding that let-7i inhibits SOCS-1 expression in DCs in response to DC activation by LPS, but not by other TLR ligands, is an indication that miRNA expression may contribute to tailoring
immune responses to pathogens (Zhang et al., 2011). The ability of miRNAs to impart DCs with both pro- and anti-inflammatory, and even tolerogenic, capacities also raises the possibility that miRNAs are involved in the discriminative responses to pathogenic and commensal bacteria by the gut immune system. Finally, in addition to providing further insights into immune regulation, an enhanced understanding of miRNA biology in DCs may also reveal novel molecular origins of immune dysfunctions or malignancies.