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Non-steroidal anti-inflammatory drugs for adjunctive tuberculosis treatment

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Tuberculosis (TB)continues to cause 1.4 million deaths annuallydespite effective treatment being available since the 1970s. The standard four-drug TB treatment regimen, of isoniazid (INH), rifampicin (RIF), pyrazinamide, and ethambutolachieves 90% cure rates in programmatic settings[1].*Mycobacterium tuberculosis* (*Mtb*) bacilli are difficult to eradicate since they exist in a spectrum of replication states from metabolically active, "rapid" replicators to nearly "dormant" non-replicating persisters. Thus, treatment is required for a duration of at least six months in two phases: two months of all four drugs in the intensive phase, and four months of RIF and INH in the continuation phase.Major challenges for TB control programs includepoor compliance, resulting in theemergence ofmulti-drug-resistant*Mtb*strains, which require the use of more toxic second line drugs for at least 24 months [1]; problems also arise from failures in drug supply and from drug intolerance. Shortening of the TB treatment period for both drug sensitive and drug resistant TB, would greatly improve TB disease management and infection control.

Immunotherapy has been considered as a possible approach toward shortening of chemotherapy, with a wide range of cytokines or their inhibitors and chemical or biological immunomodulatory compounds being explored[2]. However, the factors that determine the replication rate of *Mtb* and synergize or antagonize the effectiveness of mycobactericidal drugs remain poorly understood. Efforts at developing new drugs that act ononreplicating populations of *Mtb*, whichthrive under the influence of nitric oxide and hypoxia,identified rhodanines[3].Post-chemotherapy relapse was shown to require cyclosporine-sensitive and genetically influenced host immunity, which develops early after infection [4]. Such a relapse can be alleviated by the immunomodulatory agent

muramydipeptide [5] or by passive antibody, combined with IFNγ and anti-IL-2 treatment [6]. Recently, an anti-inflammatory drug, oxyphenbutazone, was found to be cidal against both replicating and dormant forms of*M.tb*[7].

Adjunct treatment for TBin experimental mouse modelsusing generic, non-steroidal anti-inflammatory and analgesic drugs (NSAIDs)showed that diclofenac (2-[2,6-dichloranilino] phenylacetic acid)inhibited colony forming units in spleen and liver [8]. Further synergistic activity was observed in combination with streptomycin.Aspirin and Ibuprofen(iso-butyl-propanoic-phenolic acid) (IBP) were reported to enhance the mycobactericidal effect of pyrazinamide [9].Inhibition of the synthesis of PGE_2, which is elevated in the lungs at a late phase of TB, has been shown to reduce infection [10]. This finding is plausible, considering that PGE_2 inhibits phagocytosis, bacterial killing, production of nitrite and Th1 cytokines.These immunomodulatory functions are exerted via four EP receptors [11]. In this issue of the Journal, Villaplana *et al*report that Ibuprofen, in clinical use for several decades,can alleviate the lung pathology in a special mouse model, in which the lesions are similar to TB in humans. There are several ramifications of this finding relating to the cellular targeting mechanisms and for clinical translation into improving TB treatment in humans.

NSAIDs are the most commonly used analgesic and anti-inflammatory drugs worldwide.The therapeutic effects of NSAIDs are primarily due to their ability to inhibit COX-1 and COX-2 cyclooxygenases which convertarachidonic acid to prostaglandin H_2 and prostacyclin, (which lead to release of mediators of pain, inflammation, and fever) and to

thromboxane A_2(which stimulates platelet aggregation and vasoconstriction).Ibuprofen islisted as a 'core' medicine in the http://en.wikipedia.org/wiki/World_Health_Organization*Model List of Essential Medicines* of WHO, is a widely used analgesic and anti-inflammatory drug thatinhibits both COX-1 and COX-2 cyclooxygenases. COX-1 inhibition is responsible for unwanted side effects, such as gasterointestinal ulceration and bleeding. However, even selective COX-2 inhibitory NSAIDs carry a cardiovascular risk, leading tohypertensionand myocardial infarction, due to COX-2 inhibition in vascular endothelial and smooth muscle cells [12].

Though challenging, the results obtained by Villaplana *et al*are highly relevant for further consideration of evaluation of the use of IBP in humans for the adjunct treatment of drug sensitive and drug resistant TB. The rationale for use of adjunctive anti-inflammatory therapy for TB treatment is to alleviate the excessive and harmful host inflammatory responses which lead to pathological lung lesions. Induced by antigenic and immunomodulatory glycolipid stimuli, these 'decoys'[13]trigger host reactions, which are protective in the majority, but become pathogenic in the minority of infected subjects.The lungs serve for the entry of *Mtb* infection, evasion from protective systemic host defences, persistence of latent *Mtb* infection and formation of pathological lesions, which are essential for efficient transmission of *Mtb* from the granuloma lung lesion into the alveoli and aerosols.Anti-inflammatory therapy is, thus, targeted towards lung granulomatous lesions generated by an influx of a range of immune cells such as monocytes, lymphocytes and neutrophils (PMN)[14]. All these cell types produce prostaglandins and, therefore, can be targets for the action of COX-1 and COX-2 cyclooxygenase inhibitory drugs.

Considering the pilot nature of Villaplana et al's results in C3HeB/FeJ mice, the authors restrained from eluding on the question, what is the possible target cell for IBP therapy. PMNs need to be considered, bearing in mind that their function is probablydefensive early after*Mtb* infection, but aggravating in advanced disease [15]. Therefore, the timing of IBP therapy would need to aim at the late stage and avoid interfering with the early stage of infection. The protective function has been associated with PMN granule derived peptide and cationic protein defensins, which can be cidal for both intra- and extra- cellular Mtb [16] or by the activation of macrophages. On the other hand, the pathogenic function of PMNs has recently been attributed to the IFN-inducible Programmed death-1 ligand, with the caveat, that either its excess or absence could be pathogenic[15]. The perceived need for 'balancing' indicates how difficult it may be to identify a beneficial therapeutic regimen. While the influx of PMNs to lesions is attractedbyIL-17, produced byγδT cells or Th17 cells, IL-8and by the MIGchemokine, the mechanisms resulting in PMN depletion by IBP might involve their apoptotic death and clearance by macrophages. Thus, IBP may act by either reducing the influx of PMNsor by enhancing their clearance.

Inhibition of PGE_2 synthesis by IBP may be favorable for host resistance, considering that $PGE_2$is acting against the production of IL-1,TNF-α and reactive oxygen and nitrogen intermediates by macrophages. It also inhibits IL-12 expression by dendritic cells and of IFNγ and IL- 2, while promoting the production of IL-10 and IL-4 by lymphocytes. On the other hand, COX mediated inhibition of PGE_2 synthesis in macrophages may be unfavorable for the host in the early phase after Mtb infection, since PGE_2 protects the mitochondrial

membrane against damage from *Mtb*, prevents necrosis and promotes the apoptosis of infected macrophages, which leads to both innate and adaptive immunity[17]. In fact, virulent, but not the attenuated *Mtb* evades the host response by inducing the production of lipoxin A4, which inhibits cyclooxygenase 2 production and PGE_2 biosynthesis.However, the picture seems more complex, considering that the ESAT-6 secretory antigen of Mtb stimulates PGE_2production, perhaps with the pathogen's strategy to permit initial, but impede later TLR mediated signaling [18].Finally, the possible action of IBP on alveolar epithelial cells, which also make COX cyclooxygenases, also needs to be considered.

IBP could also influence the production of Leukotriene B_4 (LTB_4), whichis an eicosanoid, elevated by*Mtb* infection, but with opposite effects than PGE_2[19]. Blockade of COX-2 by administration of the celecoxib NSAID enhanced metabolism via the 5-LO pathway, indicating that the protective effect against Mtb may be due to increased leukotriene production by alveolar macrophages and that the immunostimulant effects of LTB_4 dominate over the immunosuppressive actions of PGE_2[10]

The key open question remains, whether IBP could be beneficial as an adjunctive treatment when combined with TB chemotherapy. The mouse experimental models offer the best opportunity for further preclinical research. However, suitable conditions will need to be chosen for several aspects, such as the route and dose of infection, time and schedule of therapy and endpoint criteria, such as lung pathology, bacterial load, weight loss or survival. It is commendable that mice with human-like lung pathology features, such as the C3HeB/FeJ (used by Villaplana et al) or the I/St strain [20], develop caseating granulomas, which develop into liquefacted lesions, with a massive cellular invasion of alveolar spaces

mainly by neutrophils. In common for humans and mice, tubercle bacilli in such lesions tend to multiply extracellularly and upregulate the expression of hypoxia-associated genes. Significantly, mice with these lesions are prone to postchemotherapy relapse to a greater extent than conventional inbred mice [20]. Using low-dose aerosol infection, leading to late pulmonary pathology, might be most suitable to finding out, if IBP treatment, adjunct to chemotherapy could reduce the relapse rate.

Trials on the use of IBP as an adjunct to TB chemotherapy in humans are warranted in clinical situations with pronounced inflammatory pathogenesis. This is strongly supported by the already established routine use of corticosteroids with standard TB drug treatment for TB meningitis (TBM), brain tuberculomas and severe cases of military TB, resulting in reduced mortality from TBM[21]. Aspirin has been proven to be of use in preventing stroke and 3 month mortality in patients with TBM [22].A range of pharmacological agents with immunosuppressive and immunomodulatory activity have been used for tuberculosis-related paradoxical immune reconstitution inflammatory syndrome (TB-IRIS) after initiation of anti-retroviral therapy in HIV-infected TB patients. Adjunct therapy with steroids reduces morbidity associated with moderately severe IRIS[23].The beneficial effects of prednisone in TB-IRIS appear to be mediated via suppression of predominantly proinflammatory cytokine responses of innate immune origin, not via a reduction of the numbers of antigen-specific T cells in peripheral blood. NSAIDs may be as effective as corticosteroids for treatment of non-life threatening TB-IRIS in HIV-infected patients.

Many patients who need to move on to second-line therapy for drug-resistant TB do not receive the treatment they need because the TB drugs are too expensive. IBP and other NSAIDs are comparatively very cheap.Whilst IBP does have some potential side effects, these are likely to be acceptable when compared with the toxicity and side effects of TB drugs. Being a drug with comparatively little risk and good safety profile, the temptation is to immediately evaluate the use of IBP as an adjunct to TB treatment in humans in phase 3 clinical trials, though other evaluations may be required. Other COX-2 inhibitors with fewer side effects than IBP could be more suitable for adjunctive TB therapy.Thus, rofecoxib and clecoxib, which preferentially inhibit COX-2 resulting in better tolerability [24], should be evaluated in parallel with IBP.Ibuprofen lysine salt with increased water solubility allows intravenous use with more rapid onset of action and suitable for topical use. Could, IBP-lysine be of interest for aerosol application in TB?Of the other considerations, the timing of IBP delivery could be critical to improving treatment outcomes: if targeting PMN infiltration, than it should be given late, when the inflammatory pathology is excessive, not early when PMNs might be protective.

Case reports documented reactivation of pulmonary tuberculosis in 2 patients who had used NSAIDs [25]. A case-control study of 38 patients designed to test the hypothesis that such an association does exist found a statistically significant relation between the reactivation of latent *M.tb* infection and the use of NSAIDs [26]. Whether the association is direct, indirect or secondary remains unknown, but may involve mechanisms by which hydrocortisone has been shown to reactivate dormant TB in 'Cornell model' mice. The studies would need to take into account the i) the influence of human population genetics,

ii) the influence of commensal environmental microbiota, and iii) the compound life-time history ofimmunological priming, due to vaccinations and exposure to infectious diseases. Since all patients with active TB will be treated by standard chemotherapy, any consideration of clinical trials, using adjunctive treatment with IBP, will need to be judged as to whether it can shorten the period, leading to relapse-free cure.

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