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Perspective

Tuberculosis endotypes to guide stratified host-directed therapy

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SUMMARY

There is hope that host-directed therapy (HDT) for tuberculosis (TB) can shorten treatment duration, help cure drug-resistant disease, or limit immunopathology. Many candidate HDT drugs have been proposed, but solid evidence only exists for a few select patient groups. The clinical presentation of TB is variable, with differences in severity, tissue pathology, and bacillary burden. TB clinical phenotypes likely determine the potential benefit of HDT. Underlying TB clinical phenotypes, there are TB "endotypes," defined as distinct molecular profiles, with specific metabolic, epigenetic, transcriptional, and immune phenotypes. TB endotypes can be characterized by either immunodeficiency or pathologic excessive inflammation. Additional factors, such as comorbidities (HIV infection, diabetes, helminth infection), structural lung disease, or mycobacterial virulence also drive TB endotypes. Precise disease phenotyping, combined with in-depth immunologic and molecular profiling and multimodal omics integration, can identify TB endotypes, guide endotype-specific HDT, and improve TB outcomes, similar to advances in cancer medicine.

INTRODUCTION

With 10 million estimated annual cases, tuberculosis (TB) remains a global scourge. Following infection with *Mycobacterium tuberculosis* (*Mtb*), a minority of people progress to active TB disease, with a complex interaction of host, mycobacterial, and environmental factors determining the risk for disease progression. Active TB presents mostly as pulmonary disease, ranging from subclinical localized infiltrative disease, pleurisy, or hilar lymphadenopathy to severe or widespread disease with extensive necrosis, lung cavities and fibrosis, disseminated miliary disease, or extrapulmonary disease including osteoarticular, abdominal, spinal, and cerebral involvement that may be life threatening. Also, following treatment, some patients recover uneventfully, whereas others are slow to clear disease even with effective antimicrobial therapy or even experience paradoxical worsening of TB with progressive immunopathology.[1] Such worsening has been described as immune reconstitution inflammatory syndrome (IRIS) in the context of HIV infection but may also occur in HIV-negative patients. Finally, after successful completion of treatment, some patients experience disease recurrence. These variable presentations and outcomes of TB disease can be termed "phenotypes."

Although antibiotics target mycobacteria, there is enthusiasm that host-directed therapy (HDT), which acts by modulating host immunity or other molecular

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mechanisms, can improve TB outcomes. The potential benefit of HDT is likely to depend on TB disease phenotype and on timing of the use of HDT. Goals of HDT include clearance of *Mtb* (potentially shortening antimicrobial treatment or improving outcomes of multi-drug-resistant TB) and/or limiting damaging inflammation and immunopathology. So far, although many candidate drugs have been proposed or trialed as HDTs for TB, there is only evidence for a beneficial effect of corticosteroids for two specific narrow indications: TB meningitis (TBM) in HIV-negative patients[2] and IRIS in the context of HIV co-infection.[3,4] In addition, in patients with HIV-associated TB, antiretroviral therapy restores host immunity, improves long-term outcomes, and can be considered an effective form of HDT. One reason behind the lack of consistent evidence for other drugs is the fact that their effect may depend on disease phenotype, or on underlying biological pathways, or *endotypes*.

Although phenotype is the observable, clinical presentation, endotypes are the distinct immunologic and molecular pathophysiologic mechanisms that lead to disease.[5,6] Different endotypes can result in similar or dissimilar clinical phenotypes. For example, asthma has a relatively uniform phenotypic presentation, which can be due to eosinophilic or neutrophilic endotypes. Asthma treatment is supposed to target the specific endotype.[5,6] For example, asthma can be classified as "T2 high," "T2 low," obesity related, smoking related, or late onset; T2-high asthma is characterized by increased IgE, IL-5, IL-25, and IL-33 and is treated with inhaled monoclonal antibodies targeting IL-5.[6,7] In this perspective, we review what is known about TB endotypes, in particular the specific immune profiles and patterns of inflammatory markers (endotypes) that are regulated by a complex interplay of cellular metabolism, host genetic background, epigenetics, and gene transcriptional regulation. Other factors, especially *Mtb* genotype and bacillary load, structural lung damage, and environmental factors such as co-medication or smoking, are likely to influence host endotypes. In cancer medicine, large collaborative efforts, such as The Cancer Genome Atlas (TCGA), have helped identify distinct endotypes, leading to targeted endotype-specific therapy and improved clinical outcomes. Better characterization of distinct TB endotypes, and their underlying host-related, environmental, and pathogen-related factors, holds promise to identify TB endotype-specific therapy and improved clinical outcomes.

Leveraging host immunity to combat *Mtb*

Defects in host immunity, immune polarization due to HIV or helminth co-infection, and rare inborn genetic defects drastically increase the risk for TB disease progression. Unlike bacterial pneumonia, which is treated for 5 to 14 days with antibiotics, TB therapy requires 6 months of antibiotics for drug-sensitive pulmonary disease and 9 to 24 months for drug-resistant disease. Each year, ~1.5 million of the 10 million TB patients die. In searching to define immune correlates of protection, the immune response that protects against TB disease progression, there is hope that these efforts will also identify the means to harness host immunity to improve TB treatment outcomes.

From a therapeutic perspective, the major objective of treatment is to kill intracellular mycobacteria, without inflicting collateral damage to host organs.[8–10] Dendritic cells (DCs), macrophages, and neutrophils are the main phagocytic cells, and all can possess pro- and anti-inflammatory characteristics under specific circumstances (Figure 1).[11] Lymphocytes, both classic $CD4^+$ and $CD8^+$ T lymphocytes, as well as natural killer (NK) cells, innate lymphoid cells (ILCs), mucosal-associated invariant T cells (MAITs), and γδ T cells, produce cytokines and implement cytotoxic killing. Cytokines can generally be classified as Th1 (such as TNF, IL-2, and interferon-gamma (IFN-γ) for combating intracellular pathogens), Th2 (IL-4, IL-5, and IL-13 for combating helminths and wound

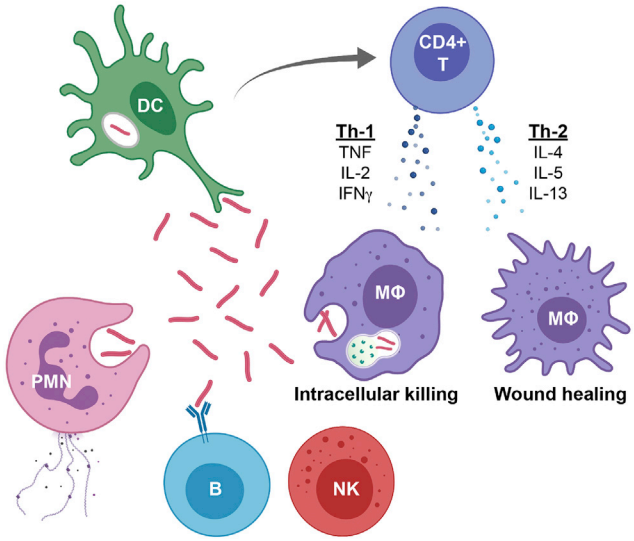

Figure 1. Overview of immune response to *Mtb* infection

1. Dendritic cells (DCs), polymorphonuclear neutrophils (PMNs), and macrophages phagocytose *Mtb*, leading to either mycobacterial survival or death.
2. DCs are professional antigen-presenting cells and bring *Mtb* antigens to the draining lymph node, where DCs implement antigen presentation and activate the classic $CD4^+$ T cell response.
3. Macrophages are the preferred intracellular niche for *Mtb*. When activated by TNF and IFN-γ, they upregulate ROS, lysosomal acidification, and phagolysosome maturation and increase *Mtb* killing capacity (more M1-like macrophages). When activated by IL-10, IL-4, IL-5, or IL-13, they downregulate these processes, temper intracellular killing, and focus on wound healing (more M2-like macrophages). Tissue macrophages represent a spectrum that typically includes elements of both M1 and M2 phenotypic characteristics.
4. Neutrophils phagocytose and kill *Mtb* but also produce cytokines, and when they die, they spew out an extracellular matrix of chromatin and proteins that alert other immune cells.
5. $CD4^+$ T cells produce cytokines, such as Th1 cytokines TNF, IL-2, and IFN-γ that stimulate macrophages for intracellular killing or Th2 cytokines IL-4, IL-5, and IL-13 that promote macrophage wound healing.
6. Cytotoxic T cells, as well as natural killer (NK) cells, MAITs (mucosal-associated invariant T cells), and innate lymphoid cells (ILCs), in addition to producing activating or suppressive cytokines, can induce perforin and granzyme-mediate cytotoxic killing of *Mtb*-infected cells.
7. B cells produce antibodies that neutralize extracellular *Mtb*, mediate NK CD16 antibody-dependent cytotoxicity, and mark *Mtb* for opsonophagocytic clearance.

healing), and Th17 (IL-17, IL-21, and IL-22 for combating extracellular bacteria and fungi). Lymphocytes, especially NK cells and $CD8^+$ T cells, also have cytotoxic killing potential; host cells infected by *Mtb* are destroyed by injection of perforin and granzymes into their membranes.[12–14] B cells, often ignored in mycobacterial immunity, aid in opsonophagocytosis and antibody-dependent cell-mediated cytotoxicity.[15] Increasing knowledge of the complex interaction of multiple immune cell types and the delicate regulation of immune homeostasis has made it clear that there is not a single immune correlate of protection, as there are multiple pathways by which an individual can progress to TB disease.

Known TB immune endotypes and the multiple immune requisites against *Mtb*

IL-12-IFN-γ up- and downstream immune-deficient endotypes

IFN-γ is the immune biomarker most often evaluated as a potential immune correlate of TB protection; yet an in-depth evaluation of IFN-γ signaling pathways highlights the heterogeneity of immune dysfunction that can lead to TB progression. The Mendelian susceptibility to mycobacterial diseases (MSMDs) are a collection

of rare genetic defects that increase an individual's risk for TB and other mycobacterial diseases.[16,17] A large number of these defects are related to IFN-γ signaling, either upstream (*IL12B2*, *IL12RB1*, *NEMO*, *IRF8*, *SPPL2a*) or downstream (*IFNGR1*, *IFNGR2*, *STAT1*, *IRF8*) of IFN-γ.[17] *IL12RB1* and *IFNGR1* mutations, representative of IFN-γ upstream and downstream mutations, highlight why a single immune correlate of protection is unlikely to be identified.[16]

Mutations upstream in the IFN-γ pathway result in decreased IFN-γ production and decreased capacity to kill intracellular bacteria (Figure 2A). The most common means to trigger the IFN-γ pathway starts with the IL-12 receptor, a heterodimer consisting of IL12RB1 and IL12RB2. Aberrant IL12RB1 mutations affect IL-12, IL-17, and IL-23 signaling.[18] Intracellular components of the IL-12 receptor interact with tyrosine kinases (JAK2 and TYK2) to transmit the IL-12 signal to STAT4. Phosphorylation of STAT4 and subsequent activation of other transcription factors, including IRF8 (interferon regulatory factor), increases expression of multiple genes involved in the host defense against intracellular pathogens (*GBP1*, *GBP2*, *CXCL9*, etc.), and in particular the production of IFN-γ (Figure 2A).[19] Upstream defects in IFN-γ signaling lead to decreased lymphocyte proliferation, poor granuloma formation, decreased CD4 memory formation, immature NK cells, and decreased intracellular killing capacity.[20,21] The major functional result of IL12RB1 and other upstream MSMD mutations is a decrease in the abundance of IFN-γ produced (Figure 2A). Production of IFN-γ results in cell biologic changes that upregulate reactive oxygen species (ROS) production, lysosomal acidification, and phagolysosome maturation, leading to increased killing of intracellular pathogens.[8,19] Treatment of upstream defects includes targeted antimicrobials augmented by adjuvant recombinant IFN-γ treatment.

In contrast, downstream defects are characterized by excess IFN-γ and a failure to transmit the signal. IFN-γ signals through the IFN-γ receptor, a heterodimer of IFNGR1 and IFNGR2. Extracellular activation of the receptor signals to the intracellular domains to activate the tyrosine kinases JAK1 and JAK2, which phosphorylate STAT1.[16] In the nucleus, pSTAT1 induces epigenetic and transcriptional changes resulting in the cell biology changes requisite for control of intracellular pathogens such as mycobacteria, endemic mycosis, and salmonellae.[16,22,23] Classically, IFN-γ responsiveness was measured by stimulating cells with IFN-γ followed by measuring TNF production. Nevertheless, this readout is limited in scope because the IFN-γ response is cell specific, resulting in increased chemokine production (CXCL9, CXCL10), improved antigen presentation (*TAP1*, *HLADR*), improved phagolysosome maturation (*GBP1*, *GBP5*), and lysosomal acidification (*PFK*, *IDO*, *AIM*, *DUOX*).[17,19,24] Clinically, and in contrast to individuals with upstream mutations, downstream mutations induce more severe disease, are not responsive to exogenous IFN-γ, and have worse outcomes after bone marrow transplantation.[16] Rarely individuals can develop auto-antibodies to IFN-γ that block downstream IFN-γ signaling, similar to a downstream defect. These individuals are at increased risk for intracellular fungal and mycobacterial pathogens.[23]

TNF-deficient endotype

Like IFN-γ, TNF is necessary but not sufficient for protection against *Mtb*, and TNF is often evaluated in parallel with IFN-γ as a biomarker.[25–28] One study demonstrated that individuals with asymptomatic infection are more likely to have *Mtb*-specific "polyfunctional" $CD4^+$ T cells that simultaneously produce TNF, IFN-γ, and IL-2.[25,29] TB-diseased individuals are more likely to have $CD4^+$ T cells producing only antigen-specific TNF, with decreases in IL-2 and IFN-γ.[25] As TNF and IFN-γ

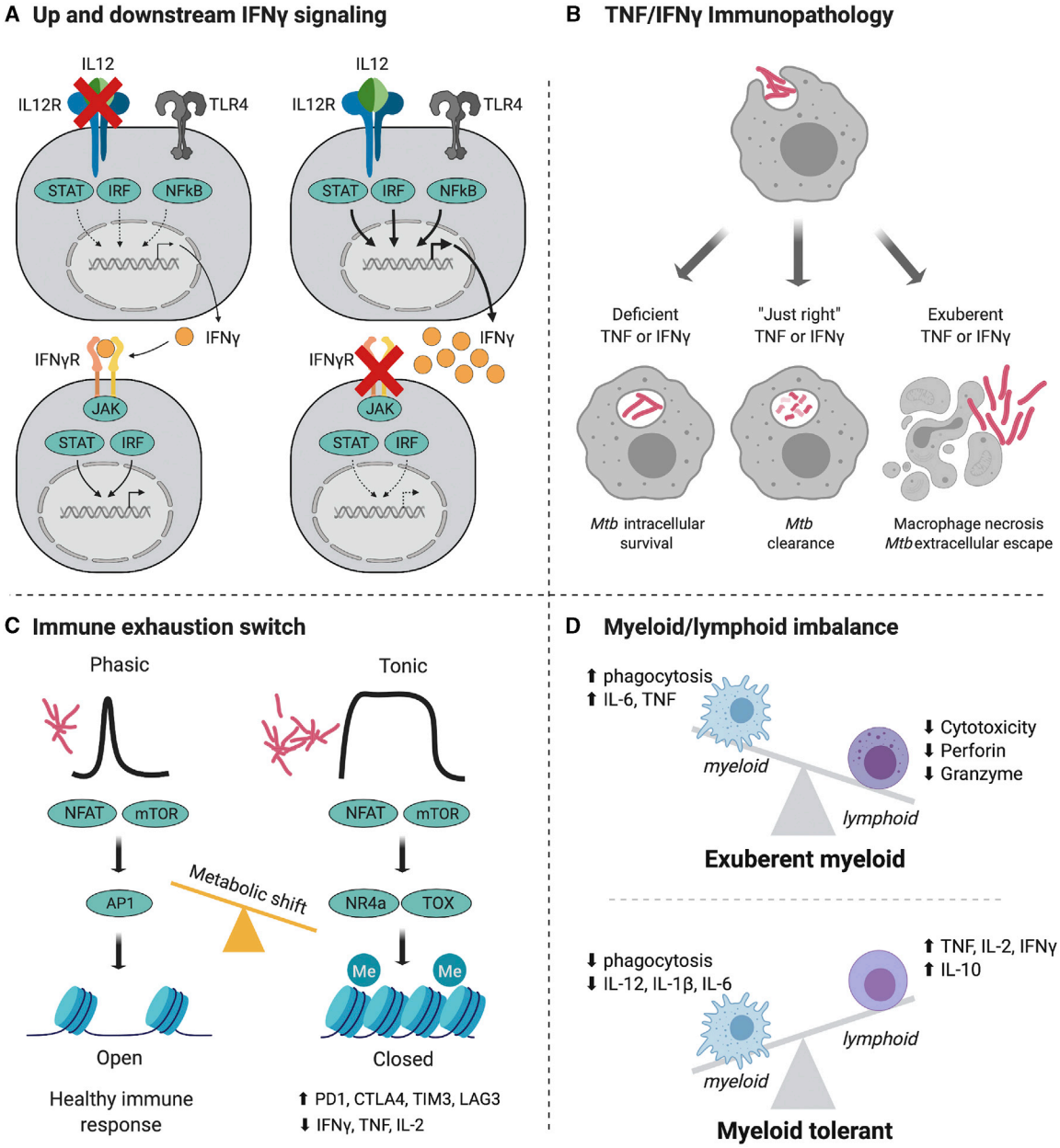

Figure 2. Endotypes are the distinct host molecular pathways by which an individual can progress to TB

(A–C) Although some endotypes are exclusive, other endotypes overlap. For example, defects in the IL-12- IFN-γ axis (A) result in immune deficiency and overlap with the IFN-γ-deficient endotype (B). Similarly, after tonic antigenic stimulation, immune exhaustion (C) leads to deficiencies in both TNF and IFN-γ. (A) IL-12-IFN-γ upstream defects in IL-12, the IL-12 receptor, IKKB, or IRF8 result in decreased IFN-γ production and decreased mycobacterial killing capacity. In contrast, downstream defects in the IFN-γ receptor STAT1 or IRF1 result in increased IFN-γ but decreased IFN-γ signal transduction and decreased mycobacterial killing capacity. (B) Host immunity has a narrow therapeutic window: deficiencies in TNF and IFN-γ result in decreased capacity to kill intracellular *Mtb*. In contrast, exuberant TNF and IFN-γ lead to macrophage necrosis and viable *Mtb* escape into the extracellular space. (C) Short antigenic stimulation induces Warburg metabolism, increased glycolysis, and glutaminolysis that triggers beneficial epigenetic immune changes. In contrast, chronic antigenic stimulation, either from TB itself or from previous HIV, helminth, or other chronic infection, results in tonic NFAT and mTOR activation, resulting in metabolic and epigenetic-mediated immune exhaustion. Immune exhaustion is characterized by decreased cytokine (TNF, IL-2, and IFN-γ) production, so this phenotype overlaps with the above.

(D) Mycobacterial immunity requires both intact and well-balanced myeloid and lymphoid immunity. Hemophagocytic lymphohistiocytosis (HLH) represents imbalance, with deficient cytotoxic T cell and NK cell immunity and myeloid-driven immunopathology with excessive TNF, IL-6, and phagocytosis. Likely, this overlaps with the exuberant phenotype depicted in (B).

act synergistically,[30,31] clarifying poly-functionality (simultaneous production of multiple cytokines by a single cell) had inspired hope for the identification of immune correlates of protection. However, there is growing skepticism of this approach, as IFN-γ is modified by both upstream and downstream signaling as described above, and as described below, TNF itself has a narrow therapeutic window.

Clinically, the critical role of TNF is evidenced by the multifold increased risk for progression to active TB among individuals treated with monoclonal antibodies antagonizing TNF.[28,32] TNF is produced by both innate and adaptive immune cells and induces profuse and pleiotropic anti-mycobacterial activity, resulting in increased phagocytosis, increased granuloma formation, and increased *Mtb* killing.[27] TNF induces the endothelial cell adhesion requisite for granuloma formation. In addition, it results in increased chemokine production, thereby recruiting immune cell to the site of infection.[33] Intracellularly, TNF induces phagosome-lysosome maturation, acidification of the lysosome, and upregulation of nitric oxide (NO) synthetase and ROS in order to augment intracellular *Mtb* killing.[34] The TNF cellular effects are synergistic with IFN-γ, with inhibition of TNF resulting in inhibition of IFN-γ-inducible phagosome-lysosome maturation and *Mtb* killing.[35] Ideally, treatment of TB patients with TNF-deficient endotypes would remove the offending TNF inhibitory agent and/or a drug perturbing TNF signaling. Immune exhaustion, discussed in detail below, inhibits TNF, and therefore strategies to reverse immune exhaustion may restore TNF.

Narrow therapeutic window and need to avoid an immunopathology endotype

Further complicating the identification of a singular immune correlate of protection against TB disease progression is the fact that many immune functional pathways and cytokines against *Mtb* have narrow therapeutic windows (Figure 2B). Host immunity must be robust enough to kill mycobacteria, but a narrow therapeutic window must be achieved to balance the killing of intracellular pathogens without inducing exuberant immunopathology. Although IFN-γ deficiency increases the risk for TB, mouse studies have demonstrated that excessive IFN-γ results in immune-induced pathology and increased mortality.[36–39] The addition of IFN-γ to healthy macrophages *ex vivo* increases *Mtb* killing capacity[40,41]; however, in animal studies, when IFN-γ production was increased (by either PD1 inhibition or cell transfer), there was increased pulmonary necrosis, escape of viable *Mtb* into extracellular spaces and death of the animals.[36,37] Results from these studies suggest that IFN-γ has a narrow therapeutic window, with excessive IFN-γ inducing immunopathology. Furthermore, the effect of IFN-γ is modulated by IL-4, IL-10, HIF1α, and other factors.[42,43] Therefore, although IFN-γ increases the capacity of macrophages to kill *Mtb in vitro*,[40,41] this cytokine alone is not a predictive biomarker of sufficient anti-*Mtb* immunity; furthermore, increases in IFN-γ are associated with increased risk for disease progression.[44,45]

Similar to IFN-γ, TNF has a narrow therapeutic window. Specifically, multiple studies have demonstrated the mechanisms by which *Mtb* is capable of evading an unbalanced immune response.[2,46] TNF is regulated by the arachidonic acid-leukotriene pathway, with leukotriene A_4 hydroxylase as a key regulator.[47] The arachidonic acid pathway is capable of producing both the pro-inflammatory leukotriene B_4 (LTB_4) and the anti-inflammatory lipoxin A^4 (LXA^4). In a zebrafish model, the heterozygous LTB_4 genotype improved *Mtb* killing, while in contrast, the homozygous CC *LTA4H* polymorphism decreased LTB_4, decreased TNF, decreased intracellular ROS upregulation, and decreased mycobacterial killing capacity.[2,46] Similarly, the homozygous TT *LTA4H* polymorphism has decreased mycobacterial killing capacity, but

through an alternative mechanism: excessive TNF leads to RIP3-mediated necroptosis (organized cell necrosis), resulting in macrophage cell death with viable *Mtb* escaping into the extracellular space.[46] Although the studies were initially performed in zebrafish, the results correlated with the benefits of steroids for humans suffering from TBM,[2,46] in which LTB_4-high patients benefited from steroids, whereas LTB_4-low patients did not.[2] TNF is also regulated by PD1, and like IFN-γ, inhibiting PD1 boosts TNF production, but this boosting can be excessive, resulting in increased *Mtb* growth.[48] Therefore, like IFN-γ, TNF has a narrow therapeutic window, with excess inducing immunopathology. Clinically, exuberant IFN-γ and TNF TB endotypes could be treated with glucocorticoids, cyclooxygenase inhibitors (aspirin, ibuprofen, and montelukast), calcineurin inhibitors (cyclosporin and tacrolimus), or mTOR inhibitors (rapamycin). Animal models fail to fully recapitulate human disease; most mouse strains have poorly formed granulomas, and zebrafish only have innate immunity.[2,49,50] However, the anecdotal evidence that TNF blockade can successfully be implemented in TB patients with steroid-refractory IRIS and that steroids are beneficial in some forms of TB lends support that exuberant immunity is detrimental in humans.

Additional evidence for the need for balanced host immunity and of the detrimental effect of exuberant immunity is hemophagocytic lymphohistiocytosis (HLH), a relatively rare manifestation of TB.[51] HLH is a devastating disorder characterized by clinical and laboratory evidence of extreme inflammation characterized by defects in cytotoxic T cell and NK cell degranulation whereby the stimulating antigen is not cleared, resulting in macrophage over-activation syndrome (MAS) with pathologic phagocytosis and pro-inflammatory cytokine secretion. IL-1 plays a key role in the pathogenesis of HLH/MAS, and IL-1 receptor blockade has shown benefit in MAS and HLH due to rheumatologic disorders and sepsis.[52] Studies in macaques[53] and anecdotal evidence also support a role for IL-1 receptor blockade in selected TB patients with hyperinflammation (van Crevel, unpublished data), but this clearly needs more evidence. Although HLH/MAS is an extreme and rare TB endotype, markers of secondary HLH (sHLH)/MAS such as hyperferritinemia, coagulopathy, and cytopenia should be checked in TB patients with unexplained hyperinflammation, and IL-1 receptor blockade or dexamethasone plus etoposide should be considered if sHLH/MAS is diagnosed.[54]

Transition from robust to exhausted immune endotype

Even excluding individuals with pre-existing immune suppression, many previously immunocompetent individuals develop TB. However, there is evidence that after extensive antigenic stimulation, the immune system will not stay robust but will transition to exhaustion. Since 1984, lymphocytic choriomeningitis virus (LCMV) infection has been the prototypical model for studying immune exhaustion.[55] These studies provide guidelines how a healthy immune response evolves and transitions toward immune exhaustion in the setting of TB (Figure 2C).

Immune exhaustion is defined by decreased cytokine production (TNF, IL-2, and IFN-γ), decreased proliferative capacity, and increased immune checkpoint markers (PD1, TIM3, and CTLA4).[56] Upon immune activation, intracellular signaling cascades are mediated by the influx of calcium, triggering the activation of multiple transcription factors, especially NFAT.[57] The phosphoinositide-3-kinase (PI3K) cascade then activates the AKT-mTOR pathway, resulting in activation of the transcription factors IRF4 and HIF1α.[39,58] Activation of mTOR, NFAT, IRF4, and HIF1α induces cellular metabolic shifts toward glycolysis and glutaminolysis and depletion of intracellular glutathione stores.[56,59–61] Initially these metabolic shifts promote immune

activation, but if persistent, increases in TCA metabolites alter the epigenetic enzymes, inducing epigenetic-mediated exhaustion.[61–63] As intracellular calcium stores become depleted, AP-1 transcription factors become dysregulated, resulting in NFAT homodimers.[64] Tonic activation of mTOR and NFAT induces NFAT, TOX, and NR4A upregulation of checkpoint inhibitors (PD1, TIM3, and CTLA4).[65–68] The nucleosome remodeling deacetylase complex (NuRD) consisting of histone deacetylases, methyl binding domain proteins (MBDs), PAC1, DNA methyltransferases (DNMTs), and the polycomb repressive complex (PRC; including EZH2) work in concert to close chromatin, inhibit gene transcription and limit immune function.[69]

We hypothesize that this is likely beneficial to prevent exuberant immune pathology, but human studies demonstrate that these inhibitory epigenetic marks persist even after removal of antigenic stimulation. For example, in human studies, despite successful antiretroviral therapy, patients with HIV infection have DNA hyper-methylation of IL-2 for 2 years post-aviremia.[70] Children with schistosomiasis have DNA hyper-methylation that lasts at least 6 months after successful de-worming.[41] In TB, DNA hyper-methylation marks are persistent for 6 months after completing successful anti-TB therapy.[71] HIV, schistosomiasis, and TB are chronic infections, with patients having antigenic stimulation for months. In mouse studies, immune exhaustion is reversible early but becomes fixed when activation is persistent.[72] Memory-like exhausted immune cells develop 1 week into continuous antigenic stimulation in mice. TB patients have a median of 87 days of symptoms before diagnosis,[73] suggesting that individuals who initially have intact immunity will transition to an exhausted phenotype when diagnosis is delayed. If TB-induced immune exhaustion follows mechanisms similar to LCMV-induced immune exhaustion, then patients with exhausted TB endotypes could be treated with mTOR, NFAT, NR4A inhibitors, DNA hypomethylating agents, and/or histone deacetylase inhibitors to block and even reverse TB-induced immune exhaustion.[56,66,74–77]

Other potential immune deficiency endotypes: Mal, GM-CSF, IL-23, and ORAI

In addition to TNF and IFN-γ, TIRAP (Toll/IL-1 receptor domain containing adaptor protein) signaling may also have a narrow therapeutic immune window. TIRAP is involved in non-canonical signaling of IFN-γ and regulation of TLR2 and TLR4 activities by regulating MAPK activation.[78] Interestingly, a heterozygous TIRAP mutation had improved *Mtb* killing capacity, despite the SS homozygous SNP having increased production of CXCL10 and TNF.[79] Other critical components of antimycobacterial immunity include GM-CSF, IL-17, IL-21, IL-23, and STIM1. It is unclear how frequently a perturbation in these mechanisms leads to disease. A mycobacteria immune-deficient endotype characterized by auto-antibodies to GM-CSF is well described in humans,[80] and the mechanisms of IL-17, IL-21, IL-23, and STIM1 TB endotypes have also been observed in mouse models.[18,81] Identifying the role these defects play in human TB requires larger studies with the capacity and resolution to identify rare endotypes. This is discussed in more detail below.

External drivers of host endotypes

Susceptibility to and clinical presentation of TB is due to the interaction of host, environment, and pathogen. HIV infection is a classic example of an external factor driving host endotype and increasing the risk for TB. Additional risks include helminth co-infection, diabetes, and airway dysfunction from smoking and air pollution. In-depth reviews of HIV[82] and helminth[83,84] perturbations on host immunity have been previously published; therefore, below we review how diabetes, airway dysfunction, and *Mtb* features affect host endotypes and increase the risk for TB progression.

Diabetes is becoming one of the most common risk factors for TB and poor TB treatment outcomes.[85,86] Macrophages from people with diabetes show decreased phagocytosis, autophagy, and antigen presentation.[87–89] In contrast to HLH, in which myeloid cells are exuberantly hyper-activated and lymphocytes are deficient, diabetes is characterized by hyper-activated T cells and hypo-activated myeloid cells.[88,90–92] Diabetics also have other factors, such as dysregulated fatty acids and lipid metabolism, vitamin D deficiency, glycosylation end products, diminished cell-signaling molecule diacylglycerol (DAG), and diminished glutathione, a key mediator of ROS signaling, which may be involved in increasing the risk for TB disease progression or worsening TB outcomes.[87,91,93–95] This remains an area of speculation, but experimental and epidemiologic data suggest that metformin, a regulator of mTOR and one of the most commonly used drugs for diabetes, may be a promising example of an endotype-specific HDT. Metformin ameliorates lung pathology and limits *Mtb* growth in mice,[96] while in humans it improves phagocytosis, increases ROS production, and inhibits TNFa and IL-1b production.[97] In epidemiological studies, metformin decreases negative TB outcomes, including cavitation, death, and disease recurrence,[9,98] and a trial evaluating metformin as HDT for TB in non-diabetic individuals is in preparation (Kornfeld, personal communication).

Cigarette smoking and indoor air pollution increase the risk for TB through airway damage, mucociliary dysfunction, and impaired anti-mycobacterial immunity.[99–101] Cigarette smoking substantially delays the time to culture conversion in patients who receive TB treatment[102]; therefore, we hypothesize that the increased duration of antigenic stimulation increases immune exhaustion. Studies are needed to evaluate this hypothesis. TB patients have rates of recurrence higher than asymptomatic household contacts, and this risk for relapse increases further with smoking.[103] The deleterious effects of air pollution and smoking on anti-mycobacterial immunity have been reviewed; in short, they induce inflammation and impair innate (macrophages, DCs, and NK cells) and adaptive immunity (T cells, B cells, and antibodies).[104,105] Of note, bronchiectasis and other mechanical airway diseases that increase airway diameter result in permanent mucociliary dysfunction. Therefore, even if host immunity normalizes, an individual with structural lung disease is likely more prone to TB progression with a lower bacillary burden. Likely, the chronic antigenic stimulation from bronchiectasis is not its own endotype but induces epigenetic-mediated immune exhaustion, as described above. Future studies are needed to clarify if mechanical airway deficiencies induce a distinct immune endotype or overlap with other endotypes.

Mtb genotype and load are likely additional drivers of TB endotypes. Even individuals with robust, well-balanced host immunity and intact airway function can progress to disease if the bacillary burden is high enough. Considering the narrow therapeutic window of host immunity described above, full clarification and interpretation of the appropriateness of host immunity requires precise knowledge regarding the bacillary burden in patients, which is not currently available with existing technology. In addition to bacillary burden, different *Mtb* strains produce different virulence factors.[106,107] There are nine distinct *Mtb* strains that differ in their mycobacterial cell wall components, their metabolic induction of host immune cells, their induction of host inflammation, their transmissibility, and their success in different regions of the world.[108] Each *Mtb* lineage contains distinct glycolipid cell wall components,[109] and we hypothesize that this is one means by which strains may differentially modulate host immunity. Evidence for this hypothesis may be supported by studies demonstrating that rabbits infected with CDC1551 (common

lineage 4 strain) induce less myeloperoxidase, less pulmonary necrosis, and less STAT1 and NFKB signaling and had lower bacillary loads than HN878 (common lineage 2, Beijing strain).[110] Other studies suggest that the HN878 strain is more likely to induce IL-4 and IL-13 and less likely to induce IL-12 and IFN-γ than the CDC1551 strain.[111] Even within lineages, reports suggest significant differential immune induction among strains. Compared with a non-MDR Beijing strain, an MDR Beijing strain induced less TLR2-mediated AKT-mTOR glycolytic metabolism and IL-1β upregulation.[112] Sequencing and molecular epidemiologic studies have demonstrated that strain-specific transmissibility and disease progression depend on environmental factors and host genotype.[113] As integrating strain sequencing and host immunity becomes more feasible, the bi-directional relationship between *Mtb* strain and host endotypes should become more clarified.

Integration of multimodal omics to clarify TB endotypes

There is compelling evidence from case reports and animal studies for the existence of multiple distinct and contradictory TB endotypes. To determine the presence of TB endotypes, including rare endotypes, a large clinical cohort with uniformly collected data is required. This does not yet exist for immune phenotyping, metabolism, or epigenetics, but multiple studies have systematically reviewed publicly available gene expression data.[114,115] We recently embarked on analyzing publicly available transcriptomics and implementing an unbiased bioinformatic clustering analysis to identify TB endotypes with distinct metabolic, epigenetic, and immune gene expression patterns.[116] The publicly available data have limited epidemiology and therefore limited analysis of the external drivers of distinct TB endotypes. Furthermore, resting gene expression data alone are not indicative of function. TB inhibits host immunity, and multiple studies have demonstrated that TB patients have elevated baseline immune activation but a failure to upregulate upon stimulation.[25,71,117,118] Immune function depends on metabolic activation upon stimulation and epigenetic confirmation, and therefore paired metabolic, epigenetic, and functional immune phenotyping studies are needed to fully characterize preliminary TB endotypes on the basis of gene expression.

Although simultaneous evaluation of all these factors may seem a daunting obstacle, many fields have developed integrative multimodal omics approaches that have advanced clinical care (Figure 3). Endotypes can be clarified by integrating these data sources with rich and longitudinal clinical information and by leveraging approaches similar to TCGA. Initiated in 2006, TCGA includes publicly available data on >40 tumor types for more than 30,000 patients.[119] Publicly available data include paired gene expression transcriptomics, copy number variation, nucleotide polymorphisms, genome-wide DNA methylation, microRNA, and protein expression. Robust and uniformly collected epidemiology and clinical outcomes are linked to each sample. By mining TCGA, researchers determined that breast cancer consists of three subtypes with different dysregulated molecular pathways that can be differentially treated.[120] Similarly, data from TCGA identified mechanisms by which cancers escape immune checkpoint therapy, thereby developing improved combination therapies.[121] A TB equivalent of TCGA could first clarify the relationship among metabolic, genetic, epigenetic, and immune phenotypes that characterize distinct TB endotypes. To clarify external drivers of specific endotypes, the data should be properly linked with epidemiology, clinical outcomes, and description of Mtb strain, similar to the CRyPTIC Consortium.[122] TB can present as paucibacillary pulmonary TB, miliary TB, cavitary TB, gastrointestinal TB, TBM, and in many other forms of extrapulmonary TB. A TB equivalent of TCGA that combines robust clinical and immune information is likely to identify immune endotype-clinical phenotype

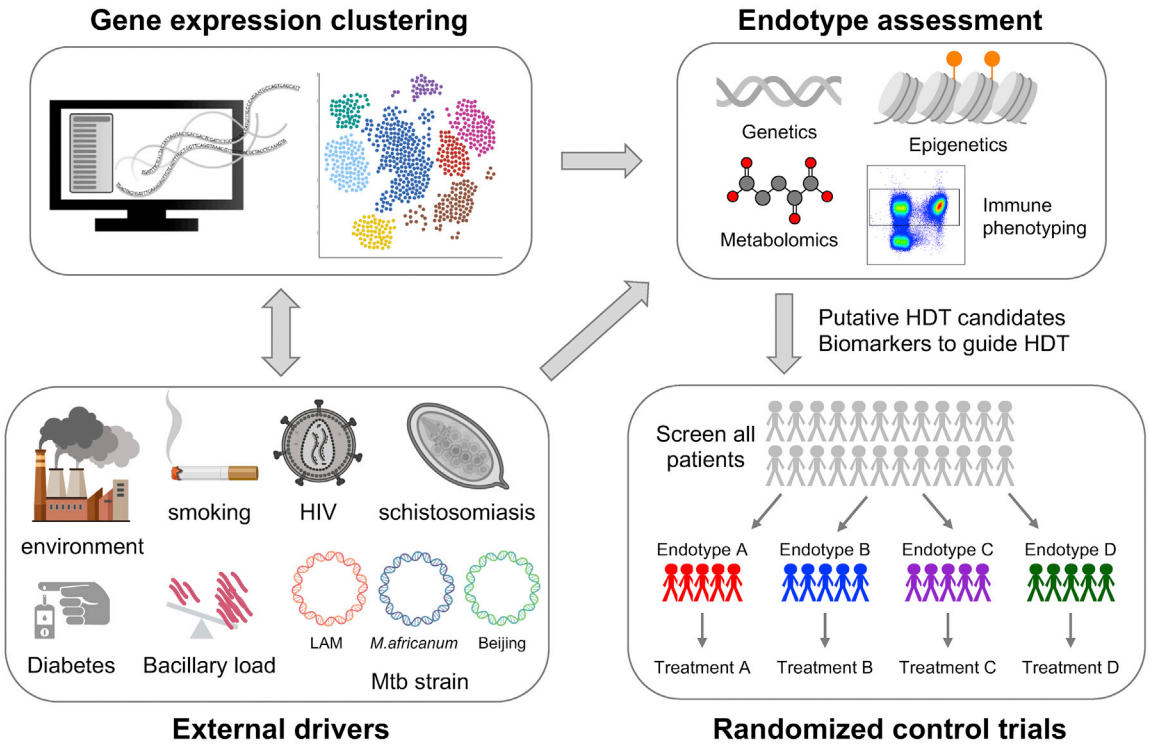

Figure 3. Unbiased clustering of publicly available data allows for identification of gene expression-derived clusters

Applying multimodal integration techniques, endotypes can be discovered and characterized on the basis of the their metabolic, epigenetic, genetic, and immune phenotype. Similarly, multimodal integration would clarify which epidemiologic factors are likely driving specific endotypes. Multimodal integration will identify the constellation of clinical epidemiology and biomarkers best suitable for treatment with putative HDT candidates that should be prospectively evaluated in umbrella and basket clinical trials.

correlations that could make implementing endotype-specific HDT easier. Clinically relevant outcomes, such as relapse-free cure, must be included to determine which endotypes require additional clinical monitoring and interventions. To date, there have been more than 20 clinical trials evaluating HDTs and more clinical trials evaluating new antibiotic regimens and shorter treatment courses. If biologic samples from these studies are contributed to a TB equivalent of TCGA, within this decade, the field should be able to identify endotype-specific treatment regimens and endotype-specific antibiotic and HDT treatment durations.

CONCLUSION

A complex interplay among host, pathogen, and environment contributes to susceptibility and clinical presentation of TB. Distinct and overlapping immunological or molecular pathophysiologic mechanism or "endotypes" likely affect TB phenotypes. We propose at least three mutually non-exclusive endotypes: one caused by IL-12-IFN-γ signaling defects, one characterized by exuberant hyperinflammation, and one by immune exhaustion. Additional factors that drive these endotypes, such as co-morbidities, structural lung damage, and *Mtb* lineage, should also be considered.

Powerful bioinformatic approaches that effectively integrate large epidemiologic and multimodal omics datasets promise to clarify these distinct TB endotypes and help identify endotype-specific HDTs. At times, endotype-specific HDT will temper exuberant immunity, while at other times it will augment immunity to improve

mycobacterial clearance. Previous trials failed to identify a benefit for HDTs such as exogenous IFN-γ,[123] but these trials were generically implemented in all TB patients without designating their endotypes or clinical phenotypes. A recent trial demonstrated that vitamin D combined with the histone deacetylase inhibitor phenylbutyrate lessened TB disease severity, in particular among patients with low vitamin D levels and moderate to severe TB disease.[124] Could supplemental vitamin D be targeted to the TB endotypes most in need? Would exogenous IFN-γ benefit IFN-γ-deficient patients but be detrimental to those with excessive IFN-γ? Similarly, TB endotypes likely determine the duration of antimicrobial therapy needed by individual patients. Recent studies using antibiotic regimens that shortened time to mycobacterial clearance were associated with increased risk for relapse.[1] These studies considered TB a uniform disease, and new studies are being developed to evaluate if certain TB clinical phenotypes could successfully be treated with shorter antimicrobial regimens.[1] Incorporating evaluation of TB endotypes, in particular persistent epigenetic-mediated immune exhaustion, is likely to help identify individuals at risk for relapse versus those who could successfully be treated with shorter regimens. For 48 years, TB treatment has not changed; applying these powerful bioinformatic tools promises to identify endotype-specific therapies and treatment durations.

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