Adding Another Piece to the Puzzle of Why NTM Infections Are Relatively Uncommon despite Their Ubiquitous Nature

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ABSTRACT

Since nontuberculous mycobacteria (NTM) are pervasive in the environment and NTM infections are relatively uncommon, underlying hereditary or acquired host susceptibility factors should be sought for in most NTM-infected patients. To facilitate identification of underlying risk factors, it is useful to classify NTM disease into skin-soft tissue infections, isolated NTM lung disease, and extrapulmonary visceral/disseminated disease because the latter two categories have unique sets of underlying host risk factors. Nakajima and coworkers (M. Nakajima, M. Matsuyama, M. Kawaguchi, T. Kiwamoto, et al., mBio 12:e01947-20, 2021, https://doi.org/10.1128/mBio.01947-20) in a recent issue of mBio found that Nrf2 (nuclear factor erythroid 2-related factor 2), a transcription factor that is induced by oxidative stress but induces antioxidant molecules, provides protection against an NTM infection in a murine model. While they showed that Nrf2 induction of Nramp-1 enhanced phagosome-lysosome fusion, we discuss other potential mechanisms by which oxidative stress predisposes to and Nrf2 protects against NTM infections.

KEYWORDS

NF-kappa B, Nramp-1, Nrf2, mycobacteria, oxidative stress
*defect in the interferon gamma (IFN-γ) axis, primary ciliary dyskinesia, prior tuberculosis, or idiopathic). However, NTM-LD may also occur in certain individuals with no known primary risk factors and NTM may be a de novo cause of bronchiectasis. Some of these individuals have minor variants of ciliary, connective tissue, cystic fibrosis transmembrane conductance regulator, and immune-related genes which, collectively via a multigenic paradigm, predispose them to NTM-LD (3). Some other associative genes that have been analyzed with increased oxidative stress in the Nrf2—vitamin D receptor.

Extrapulmonary visceral/disseminated disease most often occurs in individuals with frank immunodeficiency. Such underlying disorders may be genetic/hereditary (e.g., defect in the interferon gamma [IFN-γ]—interleukin-12 [IL-12] axis, nuclear factor-kappa B [NF-κB] essential modulator [NEMO] mutation or GATA2 mutation) or acquired (e.g., untreated AIDS, anti-IFN-γ antibody autoimmunity, or use of anti-tumor necrosis factor [TNF] agents).

Nakajima and coworkers (4) studied the role of Nrf2 (nuclear factor erythroid 2-related factor 2) in host defense against *Mycobacterium avium* complex (MAC) subsp. *hominissuis* infection in mice. Nrf2 is a transcription factor that is normally sequestered in the cytoplasm, but in an oxidative state, it translocates into the nucleus and induces phase II cytoprotective and antioxidant genes, providing negative feedback against oxidative and other cellular stress. They showed that mice with disruption of the *NFE2L2* gene, which encodes Nrf2, were more susceptible to an intranasal MAC infection with greater bacterial loads in the lungs, livers, and spleens, indicating the importance of innate immunity against NTM infections. Interestingly, there was also no difference between the wild-type and Nrf2—/— mice as measured by a DNA oxidative stress marker and a lipid peroxidation assay in the lung cells—even though one would have expected increased oxidative stress in the Nrf2—/— mice.

### TABLE 1 Some genes associated with NTM-LD

| Gene (protein) | Findings |
|---------------|----------|
| Fibrillin-2    | NTM-LD in patients with Marfanoid body habitus (taller stature, pectus excavatum, and scoliosis) are well described (34, 35). A patient with congenital contractual arachnodactyly, a genetic disorder due to FIBRILLIN-2 gene mutation and which shares many clinical features with Marfan syndrome was reported with NTM-LD (36). |
| MICA          | In 300 NTM-LD patients, the A6 allele for MICA was significantly more frequent than control subjects (37). MICA is a membrane-bound glycoprotein expressed on various cell types, including alveolar macrophages, epithelioid cells, multinucleated giant cells, intestinal epithelium, and bronchial epithelium, and engages the NK GR2D receptor on NK cells, γδ T cells, and CD8+ T cells. The A6 allele is a marker for high MICA expression on inflammatory cells and may be involved with increased tissue damage in NTM infections. |
| MST1R         | Variants of the MST1R gene, which encodes a tyrosine kinase found in the cell surface of airway epithelial cells and fallopian tubes, and involved with ciliary function, were found to be more common in NTM-LD patients than controls (3, 38). |
| TLR2          | In 80 patients with NTM-LD and 84 healthy controls from Korea, using PCR and RFLP to analyze two polymorphisms of the TLR2 gene (Arg677Trp and Arg753Gln) found none of the patients or controls possessed either one (39). Shorter GT repeats in the intron II region of the TLR2 gene was more common in NTM-LD subjects compared to controls (40). |
| VDR           | In 56 patients from United Kingdom with NTM-LD due to *Mycobacterium malmoense* in which three specific VDR polymorphisms were analyzed, there was decreased prevalence of the FokI polymorphism and increased frequency of Apal and TaqI polymorphisms (41). However, two other studies from Far East Asia found no difference in either the FokI or TaqI polymorphisms between NTM-LD and healthy subjects (12, 42). |

* Apal, *Acetobacter pasteurianus* subspecies I A; FokI, *Flavobacterium okeanokoites* I; MICA, major histocompatibility complex class I chain-related A; MST1R, gene that encodes membrane-stimulating protein receptor; NK, natural killer; RFLP, restriction fragment length polymorphism; TaqI, *Thermus aquaticus* YTI TLR2, Toll-like receptor 2; VDR, vitamin D receptor.
mice. However, not all Nrf2-mediated protection is via antioxidants, as the "Nrf2-interactome" includes hundreds of other cytoprotective genes that might not manifest in an oxidative phenotype (6, 7). Furthermore, oxidative responses vary significantly depending on the insult, and DNA damage and lipid peroxidation are indirect measures that are far downstream from the processes that generate reactive oxygen species (ROS). More sensitive methods that measure ROS directly, such as electron paramagnetic resonance (EPR) or electron spin resonance (ESR), may provide a clearer picture of the redox status in these mice (8). While they showed that the Nrf2−/− mice were more susceptible to disseminated MAC disease—as opposed to isolated lung disease—this may be due to the type of murine model used and the large MAC inoculum administered. In contrast to the findings of Nakajima et al. (4), inhibition of either Nrf2 or its downstream target molecule heme oxygenase 1 (HO-1) in THP-1 cells resulted in the inhibition of Mycobacterium abscessus, reaching the opposite conclusion that Nrf2 and reduced oxidative stress in macrophages predispose to NTM infection (9).

While we are unaware of previous studies linking abnormalities of Nrf2 to human NTM-LD, specific polymorphisms in the gene for Nramp-1 have been associated with NTM-LD, highlighting the relevance of this study to human NTM infections (10–12). However, possessing such variants does not necessarily indicate that the Nramp1 protein is defective or even if dysfunctional, whether it is clinically relevant.

In addition to the paradigm of ROS → Nrf2 → increased Nramp1 expression → increased P-L fusion, could ROS and Nrf2 affect other mechanisms that, respectively, predispose to or protect against NTM infections? Individuals with chronic granulomatous disease (CGD)—in which there is a defect in the NADPH oxidase enzyme in phagocytes with resultant defect in the formation of ROS—are predisposed to infections with Staphylococcus aureus, Gram-negative bacteria, and Aspergillus species. Even though CGD patients may be more predisposed to tuberculosis and BCG (13, 14), there is a paucity of data linking CGD to NTM infections (15, 16). In contrast, we previously showed that an antioxidant enhanced both P-L fusion and clearance of M. abscessus in macrophages (17, 18). ROS are known to either induce or inhibit various components of the NF-κB activation pathway (19). While NF-κB is important in the induction of host-protective molecules such as TNF, we have found that inhibition of NF-κB decreases A20 (a deubiquitination enzyme that inhibits autophagosome maturation by inhibiting

![Diagram showing the mechanisms by which oxidative stress—denoted by ROS—may increase susceptibility to NTM infections. ROS may decrease P-L fusion, autophagy, and NO availability. However, ROS may also activate counterregulatory signaling and metabolic pathways that reduce its own production (inhibitory lines [orange lines]), thus enhancing host immunity against NTM (inhibitory lines [red lines]). In addition to sequential activation of Nrf2, Nramp-1, and P-L fusion, ROS may also induce aerobic glycolysis that can ultimately inhibit oxidative stress as well as a primary effector cellular mechanism against NTM. Note that while lactate can induce a small burst of ROS (smaller angled arrow), this triggers Nrf2 activation to ultimately result in a net antioxidant effect. NF-κB, nuclear factor-kappa B; NO, nitric oxide; Nramp-1, natural resistance-associated macrophage protein 1; Nrf2, nuclear factor erythroid 2-related factor 2; NTM, nontuberculous mycobacteria; P-L, phagosome-lysosome; ROS, reactive oxygen species.](mbio.asm.org)
ubiquitination of Beclin-1), increases autophagosome-lysosome fusion, and helps in the clearance of MAC infection (20). However, it is important to emphasize that NF-κB activation is also important for host control of mycobacterial infections, as frank deficiency of NEMO (also known as IkB kinase-gamma, a component of the IkB kinase [IKK] complex required for NF-κB activation) predisposes to these infections (21). Since Nrf2 induces antioxidant molecules, one prediction is that Nrf2−/− mice would demonstrate increased NF-κB activation in macrophages with NTM infection, resulting in increased A20 expression, reduced autophagy, and decreased ability to clear NTM infections. Another plausible mechanism by which ROS may predispose to NTM infection is via reducing levels of antimycobacterial nitric oxide (NO) (22), as an O2 radical readily combines with NO to form peroxynitrite (ONOO−) (Fig. 1). One important caveat is that because mice produce significantly more NO than humans, endogenous NO may be significantly more protective against mycobacteria in mice than in humans. Nevertheless, Nakajima and coworkers (4) did not see evidence of increased oxidative stress based on indirect measurements in the Nrf2−/− mice compared to the wild-type mice even following MAC infection. However, other groups have measured elevated levels of ROS in Nrf2−/− mice by specific and highly sensitive methods (8); thus, it is possible that these mice do exhibit an overt oxidative phenotype that contributes to their susceptibility to NTM infection. Furthermore, since the Nrf2−/− mice are affected in utero and at birth (23), they may have adapted to oxidative stress by augmenting other antioxidative pathways by the time they reached maturity.

Nrf2 is a major transcriptional regulator of cellular metabolism and, in particular, induces a shift toward glucose metabolism (24, 25). Effective microbial defense by macrophages is linked to an infection-induced shift toward aerobic glycolysis, specifically in M. tuberculosis infection (26–28) (Fig. 1). This metabolic response resembles the “Warburg” effect known to dominate metabolism in cancer cells, where glycolysis is upregulated in the absence of an anaerobic environment. Although a direct relationship between Nrf2, microbial response, and glycolysis has not been established, activation of Nrf2 by sulforaphane, or knockout of the Nrf2 inhibitor Keap1, leads to increased glucose utilization by glycolysis, pentose phosphate shutt, and consequently, the generation of NADPH intermediates that are not only critical for maintaining redox homeostasis but also support anabolic pathways to promote cytoprotection (29). Thus, another potential explanation for impaired control and severe NTM-LD in the absence of Nrf2 could involve metabolic reprogramming. High levels of oxidative phosphorylation is reported in mycobacterial infection, with the potential to generate excess oxidative stress (30, 31). Counterregulatory (negative-feedback) mechanisms in response to the increased levels of ROS include the ability of ROS to (i) induce glycolysis which decreases ROS by less reliance on oxidative phosphorylation (32) and (ii) activate Nrf2 which induces antioxidant mechanisms, such as pentose phosphate pathway and NADPH production (Fig. 1). Interestingly, lactate generated from glycolysis has been shown to trigger a small ROS burst which then activates Nrf2 to induce a net increase in antioxidant defenses (Fig. 1) (33). Thus, while critical for the response to infection, an increase in glycolysis without effective redox balance could generate oxidative stress that is injurious to cell function, thereby impairing this basic defense mechanism.

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