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Mycobacterial TDM: A Coat to Modulate Post Primary Pathogenesis?

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Background

Trehalose 6,6'-dimycolate (TDM; cord factor) is the major external constituent comprising the waxy coat of *Mycobacterium tuberculosis*, and has been under active investigation for well over half a century. On one hand, this unique glycolipid imparts protective physical attributes to the organism, allowing survival outside of host cellular compartments. On the other hand, interaction within the host environment allows directed engagement of immunological processes advantageous both to host survival and to organism perpetuation. Classical modeling of TDM bioactivities has centered on innate immune parameters. However, recent scientific contributions shed light on additional facets that direct adaptive outcomes, warranting reexamination of TDM-induced activities that modulate manifestation of disease pathology in post primary clinical scenarios.

The characteristic histopathology associated with clinical primary tuberculosis manifests as a granulomatous lesion comprised of activated epithelioid macrophages cuffed by "ring-leader" lymphocytes [1,2]. The process allows functional sequestration of organisms with described (limited) focal inflammation, in essence providing a mechanism whereby hosts may carry the disease for later spread to other individuals. The abundant surface glycolipid antigens, mycolic acid and trehalose 6,6'-dimycolate, dictate much of the primary pathological response [3], with known granulomatous-inducing properties. In contrast, post primary tuberculosis (also referred to as secondary or adult tuberculosis) was recently redefined as a caseating pathology arising in immune competent individuals whereby organisms evade strong and effective systemic specific immunity [4-6]. In modern day cases, post primary tuberculosis is often associated with a lipid pneumonia, challenging long held beliefs that cavities arise from expansion of caseating granulomas [7].

Biological Properties Associated with Cord Factor

Cord factor was identified circa 1950 to be a petroleum ether-soluble, surfactant extractable lipid constituent of virulent mycobacteria [8-12], responsible for "cording" of organisms [13]. While original studies concluded that cord factor had toxic properties [14-16], it was discovered that extracts containing trehalose 6,6'-dimycolate (TDM) were also responsible for antigenic [17] and adjuvant activities [18,19]. However, these biological properties were soon found to be dependent upon isolation, formulation and administration methodologies. While TDM has additional properties beyond its contribution to organism morphology [20], yet direct understanding of its role in clinical disease remains elusive.

There is no perfect animal model to represent the entire spectrum of human tuberculosis disease, although there are accepted ways to mimic aspects of the primary granulomatous response [21]. Implication of TDM as a major contributor to primary tuberculosis pathology has been "validated" through use of these animal models [22-27]. There are defined molecular links for TDM to influence innate molecular responses in initiation and maintenance of granuloma related pathology [28-32], which are independent of interferon-γ [33]. What is not particularly clear is the role for TDM in post primary tuberculosis, especially in development of disease pathology regulated via adaptive lymphocytic response.

In the 1970's antibodies that were reactive to TDM containing fractions [34] were identified with the ability to modulate pathology in model systems [35], suggesting (in hindsight) that adaptive cells were involved in higher order *in vivo* reactivity. More recent investigations revealed a hypersensitive component [36] involving multiple layers of adaptive immune function that may allow TDM effective control over local microenvironments [29,37]. Structural studies theorized that conformational restraints were required for TDM to elicit many of the higher order adaptive immune functions [32,38-40]. These theories were validated in part by studies showing that removal of the cyclopropane ring alters pathology development in model systems [41-43]. Only recently have we begun to appreciate contribution of TDM to development of lymphocytic functions [44-46] and mechanisms that allow induction of TDM-specific adaptive responses [47,48]. This now gives TDM a foothold as a mediator of adaptive functions leading to post primary tuberculosis and cavitary lesion development [7].

TDM as a Mediator for Post Primary Tuberculosis

The basis for this argument has a foundation in clinical tuberculosis histopathology, from patient samples exhibiting endogenous lipid pneumonia. Reports by Hunter suggest that the rapid necrosis of tuberculous pneumonia might be due to both the activation of toxic and adaptive immunogenic properties of cord factor after contact with host lipids [4,5,7,49]. This hypothesis diverges from classical interpretation of expanding granulomas as the basis of necrotizing lesions [1,50]. Indeed, an argument may be made for a strong requirement of functional adaptive immunity in the host at the time of necrotizing conversion. The contribution of an inflammatory lipid-based process may also explain why lesions histologically appear to behave independent of one another; one lesion may progress toward cavitation while another nearby regresses. Perhaps accumulation

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of lipid in alveolar macrophages becomes the tipping point in the necrotic event. Cord factor coming off organisms *in vivo* during the inflammatory process may interact with lipids to allow both toxic and antigenic events to proceed [36,51,52].

TDM Influence on Immune Function

Host interaction with organisms must include early recognition events that mediate innate functions. Identification of TDM receptors on host monocytes strengthens these ideas. However, the direct link between interaction of TDM with these receptors on innate cells and their role in development of adaptive immunity remains unknown. Certainly this remains an open field of research towards understanding clinical transition to post primary pathology. The key to defining the role of TDM in adaptive immunity therefore must begin with understanding initial events in macrophage recognition of TDM on the surface of organisms during initial encounter. Recent discoveries link the C-type lectin Mincle [53-55] as a prime candidate receptor for TDM. This is combined, in part, by additional interactions with MARCO, TLR2 and/or CD14 which are critical for mediating activity [56] with internalized signaling events that possibly function through Card9-dependent mechanisms [57,58]. What is currently clear is that once internalized, *Mycobacterium tuberculosis* blocks maturation of phagosomes [59-61], altering molecular events critical for destruction of organisms [62] and development of intra-phagosome events critical for antigen processing [63]. TDM is directly linked to this process [64,65] and elimination of critical enzymes for TDM production [eg. attenuating fbpA) alters development of T cell immunity [66]. Indeed, over expression of these related genes products increases protective T cell response [67].

Future Studies Warranted

The role of TDM-specific lymphocytes in development of post primary tuberculosis is unclear. Critical questions remain to identify and define lymphocyte TDM-specific responses which would allow a state of lipid pneumonia to develop into full cavitary disease. While classical T cell responses may play a role, it is important to separate glycolipid responsive NKT cell functions from this group. As mentioned above, hypersensitivity to TDM can occur under appropriate conditions [44,46]. Those reports indicated that classically defined $CD4^+$ T lymphocytes specific for TDM can greatly exacerbate pathology, at least in mice. However, one would presume that NKT would be more adept at recognizing glycolipids. Yet one report elegantly describes NKT depletion in response to TDM, rather than expected proliferation [68]. However, this investigation only takes into account primary encounter with organisms. Development of post primary pathology occurs long after initial encounter with organisms, with ample time for regeneration of reactive NKT populations. Indeed, this same report indicates upregulation of CD1d (in mice) by TDM, and it has been shown that hypersensitivity to TDM requires CD1d for pathological development [44,48,69].

TDM therefore remains an exciting molecule to study, with new questions proposed on its function in development in secondary disease. We must appreciate the polar extremes of its associated biological properties. On one hand TDM is critical for protection of the organism, both outside the host and as a mediator of intracellular phagosome maturation events. On the other hand, TDM has unique properties allowing focused innate inflammatory responses to initiate a protective granulomatous response. We can now hope to add additional layers of complexity through [1] investigation of its function in regulating adaptive events in immune competent hosts, and, through [2] further understanding of physical interactions with host lipids to generate toxic cues related to development of cavitation and necrosis in post primary tuberculosis.

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