A Microbial Glycolipid Functions as a New Class of Target Antigen for Delayed-type Hypersensitivity*

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Delayed-type hypersensitivity (DTH) is marked by high levels of protein antigen-specific T cell responses in sensitized individuals. Recent evidence has revealed a distinct pathway for T cell immunity directed against glycolipid antigens, but DTH to this class of antigen has been undetermined and difficult to prove due to their insolubility in aqueous solutions. Here, glucose monomycolate (GMM), a highly hydrophobic glycolipid of the cell wall of mycobacteria, was dispersed in aqueous solutions in the form of octaarginine-modified liposomes and tested for its ability to elicit cutaneous DTH responses in bacillus Calmette-Guerin (BCG)-immunized guinea pigs. After an intradermal challenge with the GMM liposome, a significant skin induration was observed in BCG-immunized, but not mock-treated, animals. The skin reaction peaked at around 2 days with local infiltration by mononuclear cells, and therefore, the response shared basic features with the classical DTH to protein antigens. Lymph node T cells from BCG-immunized guinea pigs specifically increased IFN-γ transcription in response to the GMM liposome, and this response was completely blocked by antibodies to CD1 lipid antigen-presenting molecules. Finally, whereas the T cells increased transcription of both Th1 helper (Th) 1-type (IFN-γ and TNF-α) and Th2-type (IL-5 and IL-10) cytokines in response to the purified protein derivative or tuberculin, their GMM-specific response was skewed to Th1-type cytokine production known to be critical for protection against tuberculosis. Thus, our study reveals a novel form of DTH with medical implications.

Delayed-type hypersensitivity (DTH) is marked by high levels of memory T cell immunity directed against protein antigens (Ag). Unlike the immediate-type hypersensitivity that occurs within minutes of an Ag challenge and is associated with specific antibody responses, DTH begins to manifest several hours after an Ag challenge with the peak response reached at around 2 days and primarily involves cell-mediated immunity. The DTH response is readily elicited by an intradermal challenge with the mycobacteria-derived purified protein derivative (PPD) in sensitized individuals, such as those either infected with Mycobacterium tuberculosis, the microorganism causing tuberculosis, or vaccinated with bacillus Calmette-Guerin (BCG), an attenuated vaccine strain of Mycobacterium bovis (1, 2). Therefore, the tuberculin skin test is of medical importance not only for the diagnosis of mycobacterial infections but also for evaluation of the status of cell-mediated immunity.

Besides protein Ags, presented to T cells by MHC-encoded molecules, the list of Ags recognized by T cells has recently been expanded to include glycolipid Ags, which are presented by non-MHC-encoded molecules of the group 1 CD1 family (3–5). Human group 1 CD1 molecules (CD1a, CD1b, and CD1c) are expressed prominently in activated macrophages and dendritic cells, the two major target cell types for mycobacterial infection, and their role in eliciting T cell immunity against tuberculosis has been noted (6, 7). Furthermore, vaccination with M. tuberculosis-derived lipids and glycolipids confers protective immunity in the guinea pig model of human tuberculosis, implying pathways for host defense that are distinct from those directed against protein Ags (8). Despite advances in our understanding of the group 1 CD1-dependent T cell response to mycobacteria-derived glycolipid Ags, DTH responses to this chemical class of Ags have not been fully assessed. Because of the distinct pathways for host responses to protein and glycolipid Ags, the hypersensitivity response to glycolipids would be substantially different from that directed against proteins.

Previously, we detected skin hypersensitivity responses in sensitized guinea pigs, which were directed toward trehalose dimycolate (TDM), one of the major surface-exposed mycolyl-glycolipids expressed in the cell wall of mycobacteria (9). Although it peaked around 2 days after the challenge, the TDM-elicted response was marked by local infiltration by eosinophils rather than mononuclear cells, and therefore, the response was of a type distinct from that classically defined as DTH. Subsequent biochemical and enzymatic studies revealed that mycobacteria-derived mycoyltransferases, a family of enzymes catalyzing the final step of TDM biosynthesis, could mediate
up-regulated production of glucose monomycolate (GMM) when the microbes entered into a host where glucose was readily available as a substrate (10). This observation as well as the fact that GMM is a well-defined glycolipid Ag presented by human CD1b molecules (11, 12) prompted us to test for GMM-elicited DTH responses. By using octaarginine-modified liposomes that contain mycobacteria-derived highly hydrophobic GMM molecules, the present study found that GMM induces DTH responses that are comparable with the classically defined DTH to proteins. These results indicate that a microbial glycolipid functions as a new chemical class of Ag targeted by DTH reactions. In addition, unlike the hypersensitivity to PPD, the GMM-elicited DTH is highly skewed toward T helper (Th) 1-type cytokine production, suggesting a role in host immunity against infections by intracellular microbes, such as M. tuberculosis.

EXPERIMENTAL PROCEDURES

Purification of GMM—Chemical reagents were purchased from Nacalai Tesque (Kyoto, Japan) unless otherwise indicated. Mycobacterium avium (serovar 4) was kindly provided by Dr. Ikuya Yano (Japan BCG Laboratory, Tokyo, Japan) and grown at 37 °C in 7H9 medium supplemented with the Middlebrook ADC enrichment (BD Biosciences), 5% glucose, and 0.05% Tween 80. The bacteria were harvested when the optical density at 600 nm reached 1–1.5, and lipids were extracted with chloroform/methanol (C/M) as described (13, 14). The lipids were then dissolved in 1 ml of C/M (2:1, v/v), and 30 ml of ice-cold acetone was added. After a 20-min incubation on ice, the suspension was subjected to centrifugation at 1500 × g for 15 min at 1 °C, and the supernatant was carefully removed. The pellet was washed with ice-cold acetone, and the residue was dissolved in C/M (2:1, v/v). This was followed by fractionation by TLC using an Analtech TLC plate (Newark, DE) with a solvent system of C/M/acetone/acetic acid (90:10:10:1, v/v/v/v). The GMM fraction was extracted with C/M (2:1, v/v) from the silica gels. The preparative TLC and extraction procedure were repeated until no extra spots were detected on TLC plates.

The GMM fraction was extracted with C/M (2:1, v/v) from the lipids with a solvent system of C/M/acetone/acetic acid (90:10:10:1, v/v/v/v). This was followed by fractionation by TLC using an Analtech TLC plate (Newark, DE) with a solvent system of C/M/acetone/acetic acid (90:10:10:1, v/v/v/v). The GMM fraction was extracted with C/M (2:1, v/v) from the silica gels. The preparative TLC and extraction procedure were repeated until no extra spots were detected on TLC plates. Finally, the GMM fraction was extracted with C/M (2:1, v/v), dried, and rinsed several times with methanol at room temperature to remove any residual contamination by glycopeptido-lipids and phospholipids. The identity of the GMM preparation was confirmed by mass spectrometry. Protein contamination was not detected by silver staining of SDS-PAGE gels or by the Bradford assay. TDM was purified as described previously (14), and the M. tuberculosis-derived lipooarabinomannan was purchased from Nacalai Tesque.

Preparation of Liposomes—Stearylated octaarginine-containing liposomes were generated as described (15, 16) with slight modifications adapted for the integration of GMM. Briefly, purified GMMs in chloroform were mixed with egg phosphatidylcholine (NOF Corp., Tokyo, Japan), cholesterol (Avanti Polar Lipids, Alabaster, AL), and stearylated octaarginine (KURABO, Osaka, Japan) at a molar ratio of 7:3:0.5 in a test tube, and a lipid film was prepared by evaporating the solvent. Hydration of the lipid film was done by adding distilled water, and the total lipid concentration and the concentration were adjusted to 4 mM and 0.5 mg/ml, respectively. After the hydration, the mixture was sonicated gently for liposome formation. The efficiency of GMM integration into the liposomes ranged from 60 to 85%, as determined by TLC-based resolution of the lipids with a solvent system of C/M/acetone/acetic acid (90:10:10:1, v/v/v/v). The diameter of the liposomes was measured by dynamic light scattering, and the ζ-potential was determined by laser-Doppler velocimetry with a Zetasizer Nano (ZEN3600, Malvern Instruments, Ltd., Malvern, WR, UK). The diameter and ζ-potential of the GMM liposome were 120 ± 26 nm and 40 ± 8 mV, respectively, whereas those of empty liposome were 104 ± 10 nm and 38 ± 19 mV, respectively. TDM was incorporated into liposomes as for GMM, and its integration efficiency was 73.3%.

GMM-specific T Cell Assays—GMM-specific T cell assays using T cell receptor (TCR)-deficient Jurkat cells (J.RT3) reconstituted by transfection with GMM-specific, CD1-restricted TCRs (J.RT3/LDN5) have been described (17, 18). Human monocyte-derived dendritic cells and the C1R human B-lymphoblastoid cell line stably transfected with CD1b (C1R/CD1b), used as Ag-presenting cells in these assays, have also been described previously (17–19). The GMM liposome and the control (empty) liposome were dispersed in culture media just before use, whereas the purified GMM preparation was dispersed in the culture medium by sonication as in previous studies (17, 18, 20–23). The TCR-reconstituted J.RT3 cells (5 × 10⁶/well) were cultured with irradiated Ag-presenting cells (5 × 10⁶/well) in wells of 96-well, flat-bottomed microtiter plates in the presence of phorbol myristate acetate (10 ng/ml) and the indicated concentrations of Ag preparations. After 20 h, aliquots of the culture supernatants were collected, and the amount of IL-2 released into the supernatants was measured with the IL-2 ELISA kit (BD Biosciences).

Animals and Skin Tests—Three-week-old female Hartley guinea pigs were purchased from Japan SLC, Inc. (Shizuoka, Japan) and housed under specific pathogen-free conditions. The vaccine strain BCG Tokyo 172 (Japan BCG Laboratory) grown in 7H9 medium was harvested at its mid-log phase growth, and the viability was >70%. The bacteria were injected intradermally (5 × 10⁷ colony-forming units per animal), and 6 weeks after infection, the skin on the left flank of guinea pigs was gently shaved and depilated for skin tests. Liposome containing 5 µg of GMM as well as an equivalent amount of empty liposome was dissolved in 100 µl of PBS and injected intradermally. A skin test with PPD (0.5 µg/site, Japan BCG Laboratory) was also performed in parallel. After the injection, the skin response was assessed at the indicated time points by measuring the distance across the skin induration. Experiments were repeated twice to confirm the reproducibility of the results. All animal experiments were performed according to institutional guidelines on animal welfare and the humane treatment of laboratory animals.

Histochemistry—The excised skin samples were fixed for 1 day with 4% paraformaldehyde, dehydrated, and embedded in paraffin. The tissue sections were stained with the hematoxylin and eosin solution (Merck) and observed under a microscope. Separately, some skin samples were deep-frozen in OCT compound (Sakura Finetechnical Co., Tokyo, Japan), and the cryosections were labeled with either mouse monoclonal antibodies

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(mAbs) to guinea pig CD8 molecules (clone CT6; AbD Serotec, Oxford, UK) or those specifically recognizing guinea pig helper/inducer T cells (clone CT7; AbD Serotec) followed by incubation with FITC-conjugated donkey anti-mouse IgG Abs (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). After being washed, the labeled sections were mounted with Vectashield mounting medium (Vector Laboratories, Burlingame, CA) and viewed under a fluorescence microscope. Positive cells were counted in three randomly selected high power fields.

Cytokine mRNA Expression in GMM-stimulated Lymph Node Cells—Inguinal lymph node cells were isolated from BCG-immunized guinea pigs. The cells (2 × 10^6/well) were placed in wells of 96-well tissue culture plates and stimulated with either GMM in liposomes (1 μg/ml), TDM in liposomes (1 μg/ml), empty liposomes, lipoolarabinomannan (1 μg/ml), or PPD (0.5 μg/ml) in RPMI1640 medium (Invitrogen) supplemented with 10% heat-inactivated FCS (HyClone, Logan, UT). In some experiments the culture was performed in the presence of either the CD1F2/6B5 mouse mAb to guinea pig pan-group 1 CD1 molecules (24) or an isotype-matched control mAb (P3) (25) at a concentration of 10 μg/ml. After 18 h at 37 °C, the cells were harvested, and total RNA was extracted using the RNeasy mini kit (Qiagen, Hilden, Germany). The first-strand cDNA was synthesized from 0.1–0.5 μg of total RNA using oligo(dT) and the PrimeScript reverse transcriptase (Takara Bio, Inc., Otsu, Japan). To amplify specific transcripts, the samples were subjected to PCR for 35 cycles of 1 min at 94 °C, 1 min at 60 °C (63 °C for IL-5), and 1 min at 72 °C followed by a 10-min incubation at 72 °C. The primers used were: 5′-CTA GCT ACT ACT GCC AGT CAA GAT-3′ (sense) and 5′-GCT CTA AAA CAG CAT CGT AGT CCT-3′ (antisense) for IFN-γ; 5′-CCA TGA GCA CAG AAA GCA TGA TCC G-3′ (sense) and 5′-GCA TGG TAC CAG AGT A-3′ (antisense) for TNF-α; 5′-CTA CCA TGA GGG TGC TGC TGC AGT TGG G-3′ (sense) and 5′-CTC ACA GGC AAA ACC CAG TCT GA-3′ (antisense) and 5′-TCA CCT GCT CCA CTG CCT TG-3′ (antisense) for IL-10. The PCR products were resolved on 1.2% agarose gels and visualized by staining with ethidium bromide and UV transillumination. The experiments were repeated at least twice to confirm the reproducibility of the results.

Statistical Analysis—The statistical analysis was performed using Student’s t test. p values of < 0.05 were considered statistically significant.

RESULTS

Increased Efficiency of GMM Presentation after Incorporation into Liposomes—The GMM produced by pathogenic mycobacteria contains mycolic acids with extremely long carbon chains (C_{40-40}) and can be dissolved in chloroform. In T cell assays performed in previous studies, including ours (17, 18, 20–23), GMM was forced to disperse temporarily into aqueous media by sonication, but because of its highly hydrophobic properties, it is possible that the form of GMM prepared by this method may not be utilized efficiently for the presentation of Ags. Therefore, we incorporated GMM into liposomes constructed by defined phospholipids, cholesterol, and stearoylated octaarginine. A TLC-based analysis revealed that the GMM liposome (Fig. 1A, lane 1), but not empty liposome (lane 2), contained a lipid (indicated with a bracket) that comigrated with the reference GMM (lane 3), confirming the integration of GMM. We then compared Ag presentation efficiency of the GMM liposome with that of the sonicated GMM preparation used frequently in previous studies (10, 18, 21, 22, 26). In the presence of monocyte-derived dendritic cells, the J.RT3/LDN5 cells expressing GMM-specific, CD1b-restricted TCRs secreted IL-2 in response to the sonicated GMM preparation in a dose-dependent manner (Fig. 1B, closed circles). Strikingly, an ~10-fold more efficient T cell activation was observed when GMM was applied in the form of liposomes (closed circles), whereas no T cell activation was detected when the empty liposome was added (closed triangles). GMM incorporated in liposomes was also presented efficiently by a CD1b-transfected B-lymphoblastoid cell line (C1R/CDB1) (Fig. 1C, closed circles) that was of a cell type relatively inefficient in utilizing the sonicated GMM preparation (open circles). These results as well as its high solubility in aqueous solutions suggested that the GMM liposome might be useful for eliciting specific T cell responses in vivo.

Skin DTH Reactions Elicited by the GMM Liposome—In our previous study (9), in which TDM-elicited eosinophilic skin hypersensitivity was demonstrated in mycobacteria-infected guinea pigs, the intradermal administration of GMM in mineral
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The skin response to the GMM liposome (Fig. 3J) in these animals was also associated with a mononuclear cell mobilization that was apparently indistinguishable from the PPD-elicited response. It should also be noted that no significant histological changes were induced in the empty liposome-challenged skin of BCG-vaccinated guinea pigs (Fig. 3F) or in the GMM liposome-challenged skin of mock-vaccinated guinea pigs (Fig. 3J), suggesting that the GMM liposome-elicited response in BCG-vaccinated animals requires both BCG sensitization and the GMM challenge to occur.

Guinea pig T cells were roughly subdivided into CT7+ helper/inducer T cells and CT6+ killer T cells, based on their reactivity to commercial mAb clones, CT7 and CT6, respectively (9, 27). We carried out immunohistological analyses with these mAbs (Fig. 3, panels on the right) and determined the number of these cells in the challenged skin (Fig. 4). The absolute number of CT7+ cells was significantly increased in the GMM-challenged skin of BCG-vaccinated animals as compared with that in the GMM-challenged skin of mock-vaccinated animals and in mock-challenged skin of BCG-vaccinated animals (Fig. 4A). The absolute number of CT6+ cells was also increased significantly in the GMM-challenged skin of BCG-vaccinated animals as compared with the relevant controls (Fig. 4B). An increase in the number of CT7+ and CT6+ cells was also observed in the PPD-challenged skin of BCG-vaccinated animals (Fig. 4, A and B), and therefore, no apparent differences were observed in the infiltrating T cell subsets for the PPD-elicited and GMM-elicited skin hypersensitivity.

The Group 1 CD1-dependent Response to GMM in BCG-vaccinated Guinea Pigs—GMM-specific T cell activation depends on the function of CD1b or related group 1 CD1 molecules in humans (5), monkeys (28), and cows (29). Therefore, it would be reasonable to speculate that the GMM-elicited response in BCG-vaccinated guinea pigs was restricted to guinea pig group 1 CD1 molecules. To address this, mononuclear cells were obtained from the draining lymph nodes of BCG-vaccinated guinea pigs and stimulated in vitro with the GMM liposome in the presence or absence of the CD1F2/6B5 anti-pan guinea pig group 1 CD1 mAb. This was followed by detection of IFN-γ transcription by RT-PCR. Prominent transcription of IFN-γ was detected when the cells were stimulated with the GMM liposome, but not with the empty liposome, and the GMM liposome-elicited IFN-γ response was totally abrogated when the cells were cultured in the presence of the CD1F2/6B5 blocking mAb but not the isotype-matched control mAb (Fig. 5A). Thus, these results confirmed that the GMM-specific response induced in the BCG-vaccinated guinea pigs was dependent on the function of group 1 CD1 molecules.

Highly Biased Th1-type Cytokine Expression Elicited by GMM—It has been established that Th1-type cytokines, such as IFN-γ and TNF-α, are critical for host defense against tuberculosis, and Th2-type cytokines may potentially counteract the Th1-type responses to aggravate the disease (30). To gain insights into the significance of the GMM-elicited hypersensitivity, mononuclear cells isolated from the draining lymph nodes of BCG-vaccinated guinea pigs were stimulated with specific Ags, and cytokine profiles were determined by RT-PCR.
PPD-stimulated cells showed increased levels of not only Th1-type cytokines, IFN-γ and TNF-α but also Th2-type cytokines, IL-5 and IL-10 (Fig. 5B, right panels). In sharp contrast, only the Th1-type cytokines were detected in cells stimulated with GMM (Fig. 5B, left panels). These results suggest that, as compared with the classical DTH directed against PPD or the tuberculin skin test, the GMM-elicited DTH is more biased toward Th1-type responses. Furthermore, the lymph node cells stimulated with other mycobacteria-derived glycolipids, TDM and lipoarabinomannan, up-regulated the expression of IL-10 (Fig. 5C), highlighting the unique capacity for GMM to elicit highly Th1-skewed responses.

**DISCUSSION**

We have previously detected mycobacteria-specific, group 1 CD1-restricted “memory-type” T cell responses in BCG-vaccinated human individuals and guinea pigs (26, 27). The existence of lipid-specific memory-type T cells is also supported by the finding that vaccination with mycobacteria-derived lipids confers resistance to tuberculosis in guinea pigs (8). Nevertheless, the hypersensitivity to mycobacteria-derived lipid components that is comparable with the DTH to PPD, a hallmark of mycobacterial infection in immunocompetent individuals, has been poorly understood. Initially, we observed the TDM-elicited eosinophilic hypersensitivity that was distinct from the classical DTH response to protein Ags and failed to detect apparent hypersensitivity reactions to GMM dissolved in mineral oil (9).

With the alternative use of the GMM liposome (Fig. 1), however, the present study now detects a DTH response to GMM that is comparable both in time course (Fig. 2) and in infiltrating cell types (Figs. 3 and 4) to the classical DTH reactions to protein Ags. Therefore, the present study identifies CD1-presented microbial glycolipids as a new chemical class of target Ags to which DTH is directed. Furthermore, given the apparently skewed Th1-type cytokine response as compared with the response to PPD (Fig. 5), the DTH reaction representing high levels of GMM-specific T cell responses could potentially function as an alternative and even more accurate indicator for cell-mediated immunity against tuberculosis. Mycobacteria-derived lipid Ags presented by CD1 molecules are highly hydrophobic, and therefore, the T cell response to these Ags has been difficult to prove in vivo due to their insolubility in aqueous solutions. The present study successfully utilized octaarginine-modified liposomes that had high affinity to cellular membranes and were efficiently internalized by a variety of cells (16, 31). Moreover, the GMM liposome was designed for efficient delivery to lysosomes, where Ag loading onto CD1b molecules was proposed to occur (32), by using non-fusogenic lipids, such as egg phosphatidylcholine and cholesterol. Thus, the liposome design used in this study will be useful for the development of lipid-based vaccines and skin tests as proposed below.

Pathogenic mycobacteria grown in standard culture media produce a large quantity of TDM with only a scarce amount of
GMM, and therefore, TDM has been proposed as a major surface-exposed mycolylglycolipid in a prototypic model of the cell wall of mycobacteria (33). Immunologists, however, predict that this model may not represent pathogenic mycobacteria interacting with the host because TDM-rich mycobacteria would be easily detected and eliminated by innate immune cells expressing specific receptors, such as Mincle (34). Our recent study (10, 35) determining the pathway of GMM biosynthesis provided a clue to this enigma. Mycolyltransferases, or Ag85, mediate the final step of TDM biosynthesis by transferring a mycolate group from trehalose monomycolate captured at the donor site to trehalose monomycolate at the acceptor site. In glucose-rich environments, such as those in the host, glucose competitively gains access to the acceptor site of the enzymes and acquires a mycolate group transferred from the donor trehalose monomycolate, resulting in the biosynthesis of GMM and down-regulation of TDM production. Given that GMM is much less potent than TDM in stimulating innate immune cells (10), the switch from TDM to GMM biosynthesis by utilizing pre-existing enzymes and borrowing host-derived glucose could quickly allow the microbes to avoid the host innate immune system. GMM-expressing mycobacteria that have broken the frontline of defense mediated by innate immunity are then faced with adaptive immunity directed against GMM. A human GMM-specific T cell line, LDN5, is capable of recognizing mycobacteria-infected dendritic cells and eliminating the infected cells in a Fas-dependent fashion (7). Furthermore, as demonstrated in the present study, high levels of specific T cell responses are evoked after sensitization, which are associated with the production of IFN-γ and TNF-α, the two major cytokines that control mycobacterial infections. These findings as well as the observation that the GMM-specific T cell response preferentially occurs in live infections raise the possibility that the DTH response to GMM participates in host immunity against tuberculosis.

Obviously, an important extension of this line of study would be the development of lipid-based subunit vaccines, such as GMM, against human tuberculosis. CD1 genes lack the polymorphisms seen for MHC genes (36), and therefore, the emergence of specific haplotype-associated low responder populations would be minimized with lipid vaccines. Related to vaccine development, the issue of whether the "GMM skin test" could substitute for the tuberculin test should also be addressed from a variety of medical and pharmaceutical standpoints. Par-

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FIGURE 4. Increased numbers of CT7+ and CT6+ T cells at the site of the GMM-elicited DTH. Frozen sections of the empty liposome-challenged skin, the GMM liposome-challenged skin, and the PPD-challenged skin in BCG-vaccinated as well as mock-vaccinated guinea pigs were stained with either the mAb CT7 for detection of helper/inducer cells or the mAb CT6 for detection of killer T cells. The numbers of CT7+ cells (A) and CT6+ cells (B) were determined by counting positively labeled cells in three randomly selected high power fields (*, p < 0.01).

FIGURE 5. In vitro response of BCG-vaccinated guinea pig-derived lymph node cells to the GMM liposome. A, draining lymph node cells were isolated from BCG-vaccinated guinea pigs and stimulated in vitro with either the empty liposome or the GMM liposome. GMM stimulation was also performed in the presence of either the CD1F2/685 anti-pig guinea pig CD1 mAb or an isotype-matched control mAb (clone P3). After 18 h, the cells were harvested, total RNA was extracted, and RT-PCR was performed to detect IFN-γ transcription. B, the lymph node cells were stimulated in vitro with either the GMM liposome or PPD, and RT-PCR was performed as in A to detect the transcription of Th1-type cytokines (IFN-γ and TNF-α) and Th2-type cytokines (IL-5 and IL-10). C, the lymph node cells were stimulated with either the GMM liposome, the TDM liposome, or lipoarabinomannan, and RT-PCR was performed to detect the IFN-γ and IL-10 transcription.
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particularly, it has been proposed that a positive tuberculin test does not necessarily correlate with resistance against tuberculosis, limiting the value of the tuberculin test for diagnosis, and therefore, a critical issue to address is whether a positive GMM skin test can function as an accurate and convenient indicator for established host immunity against human tuberculosis. The present study unravels previously undefined tuberculosis-associated DTH responses that may have important medical implications.

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