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*J Immunol* 2013; 190:278-284; Prepublished online 28 November 2012;
doi: 10.4049/jimmunol.1201667
http://www.jimmunol.org/content/190/1/278

**Supplementary Material**
http://www.jimmunol.org/content/suppl/2012/11/29/jimmunol.1201667.DC1

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Lipoproteins Are Major Targets of the Polyclonal Human T Cell Response to *Mycobacterium tuberculosis*

Chetan Seshadri,* Marie T. Turner,† David M. Lewinsohn,‡ D. Branch Moody,* and Ildiko Van Rhijn*§

Most vaccines and basic studies of T cell epitopes in *Mycobacterium tuberculosis* emphasize water-soluble proteins that are secreted into the extracellular space and presented in the context of MHC class II. Much less is known about the role of Ags retained within the cell wall. We used polyclonal T cells from infected humans to probe for responses to immunodominant Ags in the *M. tuberculosis* cell wall. We found that the magnitude of response to secreted or cell wall intrinsic compounds was similar among healthy controls, patients with latent tuberculosis, and patients with active tuberculosis. Individual responses to secreted Ags and cell wall extract were strongly correlated (*r*² = 0.495, *p* = 0.001), suggesting that T cells responding to cell wall and secreted Ags are present at similar frequency. Surprisingly, T cell stimulatory factors intrinsic to the cell wall partition into organic solvents; however, these responses are not explained by CD1-mediated presentation of lipids. Instead, we find that molecules soluble in organic solvents are dependent upon MHC class II and recognized by IFN-γ-secreting CD4+ T cells. We reasoned that MHC class II–dependent Ags extracting into lipid mixtures might be found among triacylated lipoproteins present in mycobacteria. We used *M. tuberculosis* lacking prolipoprotein signal peptidase A (bpsA), an enzyme required for lipoprotein synthesis, to demonstrate loss of polyclonal T cell responses. Our results demonstrate the use of bacterial genetics to identify lipoproteins as an unexpected and immunodominant class of cell wall–associated Ags targeted by the polyclonal human T cell response to *M. tuberculosis*. The Journal of Immunology, 2013, 190: 278–284.

*Mycobacterium tuberculosis* remains a leading cause of death worldwide, and CD4-restricted T cell responses have been shown to be critical to controlling infection in both humans and animal models (1, 2). Human tuberculosis occurs along a spectrum ranging from latent infection, in which asymptomatic patients are infected with the bacillus, to active disease, in which patients can transmit the infection to others. Defining the immunodominant targets of responding T cells during natural infection has resulted in major advances in immunodiagnostics as well as new vaccines. For example, the 6-kDa early secreted antigenic target (ESAT-6) and 10-kDa culture filtrate Ag (CFP-10) are core components of QuantiFERON-TB and T-SPOT. TB, two tests approved by the Food and Drug Administration for the diagnosis of latent tuberculosis infection (3). Members of the Ag 85 complex are immunodominant Ags for T cells included in at least two subunit vaccines currently in phase II clinical trials (4). ESAT-6, CFP-10, and Ag 85 are among many highly abundant secreted proteins present in culture supernatants that have been the focus of important and productive research for >20 y (5–7). However, secreted protein Ags are only a subset of the antigenic pool available to human T cells. Many mycobacterial proteins are embedded in the cell wall, such as proteins that have undergone posttranslational glycosylation and acylation (8, 9). Further, recent studies have also shown that the lipid-rich cell wall of *M. tuberculosis* contains mycobacterial lipid Ags presented to T cells in the context of CD1a, CD1b, and CD1c (10–15). Nearly all prior screens for immunodominant Ags emphasize proteins as a source of Ags for T cells, but the discovery of lipid Ag presentation provides a rationale to consider lipidic stimuli of T cells as well. It is currently not known which *M. tuberculosis* cell wall–associated Ags are most commonly targeted by T cells from infected humans.

To address this question, we used an ex vivo assay to study polyclonal human T cell responses to *M. tuberculosis* cell wall–associated Ags. As contrasted to Ag screens carried out in small animal models after experimental infection, this approach emphasizes immune responses generated in natural infections and uses human APCs and T cells that reflect restriction by genetically diverse MHC proteins, nonpolymorphic group 1 CD1 proteins, and other species-specific aspects of the human APC–T cell interaction. We avoided T cell cloning strategies because long-term in vitro culture can induce bias and functional drift in ways that reflect the ability of individual clones to survive rather than their effects occurring directly ex vivo. To capture the clinical complexity of human tuberculosis, we studied healthy controls.
patients with latent tuberculosis infection, and patients with active disease. 

Contrary to the general view that most or all T cell–stimulating factors are secreted, we found strong T cell responses to extracts of molecules embedded within the cell wall. Human polyclonal T cell responses to these factors were strongly correlated with two secreted Ags, ESAT-6 and CFP-10, indicating T cells responding specifically to cell wall intrinsic Ags are present at high frequency. Isolation of T cell stimulatory factors from the cell wall showed that stimulatory substances were enriched with solvents designed to capture lipids. After initial studies ruled out a role for CD1, we demonstrated polyclonal CD4+ T cell responses were dependent on MHC class II, blocked by chloroquine treatment, and absolutely dependent upon lipoprotein production by M. tuberculosis. Our data reveal that cell wall lipoproteins are major targets of the human T cell response to M. tuberculosis and invoke new models regarding the role of lipoproteins as both adjuvant and T cell immunogen.

Materials and Methods

**Bacteria and Ags**

*Mycobacterium bovis* bacillus Calmette-Guérin and *M. tuberculosis* H37Rv were cultivated in 7H9 medium (Difco) supplemented with 0.05% Tween-80 and 1% glucose. Cell wall extracts were generated by exposing PBS-washed cell pellets to chloroform/methanol (2:1, v/v) followed by chloroform/methanol (1:2, v/v) at 20˚C for 2 h (Fig. 1A). Subfractions of cell wall extracts were generated by first loading 20 mg onto an open 2 × 20 silica column (Supelco), and then serially eluting with 40 ml chloroform, 40 ml acetonitrile, and 40 ml methanol. For analytical TLC, 150 μl PBS was loaded on a 20 by 20 cm2 Silica 60 TLC plate (Merck) and charred for 1 h at 140˚C in an oven. *M. tuberculosis* strain H37Rv with the lipoprotein signal peptidase gene (*ispA*) deleted and complemented have been described previously (16). Prior to enzymatic treatment or cellular assays, Ags were dried onto a glass surface under a stream of sterile nitrogen gas. Ags were then sonicated for 2 min in a water bath sonicator (Branson) in the desired aqueous buffer or tissue culture medium. Overlapping peptide pools of ESAT-6 and CFP-10 were provided by Aijit Lalvani.

**Digestion of cell wall extracts**

Pronase (Roche) and proteinase K (Sigma) were used to digest *M. tuberculosis* methanol fraction in protease buffer (10 mM CaCl2, 10 mM HEPES buffer, 25 mM ammonium bicarbonate) for 4 h at 40˚C, followed by 10 min of inactivation at 85˚C as described previously (17). Mock treatment was performed in the same buffer and at the same temperatures, but without addition of the proteases. Lipase treatment was performed using dry lipase (Roche) at 37˚C for 12 h, followed by addition of lipoprotein lipase (Sigma). The digest was performed in 100 μl PBS containing 0.25 mg/ml cell wall extract and 0.5 mg/ml lipoprotein lipase. After overnight incubation at 37˚C, the enzyme was heat inactivated for 10 min at 70˚C.

**Cellular assays**

PBMCs were separated by Ficoll density gradient centrifugation. Monocytes were isolated by adherence to plastic and treatment for 72 h with GM-CSF (300 IU/ml) and IL-4 (200 IU/ml) (Peprotech) to generate autologous monocyte-derived dendritic cells (DCs) expressing CD1. Nonadherent cells (called PBMCs) and DCs were frozen separately until use. Ag specificity and restriction were tested using DCs (5 × 104 cells per well) or CD1a-, CD1b- or CD1d-transfected K562 cells (2 × 104 cells per well) as APCs in RPMI 1640 medium supplemented with 10% (v/v) PBS (HyClone), essential and nonessential amino acids (Life Technologies), penicillin-streptomycin (Life Technologies), and 2-mercaptoethanol. For IFN-γ ELISPOT assay, cocultures of APCs and T cells were incubated for 20 h in a Multiscreen-IP filter plate (96 wells; Millipore) coated with anti-IFN-γ according to the manufacturer’s instructions (Mabtech), in the presence or absence of the mAbs that block MHC class II (L243), MHC class I (W6/32), and isotype control (P3), at a final concentration of 10 μg/ml. Overlapping peptide pools of ESAT-6 and CFP-10 were dissolved in DMSO and tested in combination at a concentration of 10 μg/ml. Total cell wall extracts and subfractions were tested at a concentration of 5 or 10 μg/ml. For TLR2 blocking experiments, DCs from a donor with latent tuberculosis were preincubated in the presence of 15 μg/ml anti-TLR2 blocking Ab or isotype control [clones T2.5 and T2.13, respectively, of Carsten Kirshning (18)] for 1 h at 37˚C. Subsequently, PBMC and methanol eluates (10 μg/ml) were added for overnight incubation prior to IFN-γ ELISPOT assay. For Ag processing experiments, 25 μM chloroquine (Sigma) was added to DCs for 15 min prior to adding Ag and T cells. Chloroquine was continuously present for the duration of the ELISPOT assay.

**Flow cytometry**

For isolation of T cell subsets, PBMCs were sorted using a FACS Aria flow cytometer using CD3 APC–Alexa Fluor 750 (Invitrogen), mAb TCR FITC (BD Biosciences), CD4 APC (BD Biosciences), and CD8 PerCP–Cy5.5 (BD Biosciences). Total T cells were isolated by sorting for CD3 only.

**Human subjects**

Patients were recruited from the Lemuel Shattuck Hospital (Jamaica Plain, MA) and from employee health services at the Brigham and Women’s Hospital (Boston, MA). This work was approved by the Lemuel Shattuck Hospital and the Partners Healthcare Institutional Review Boards. Our study population consisted of three subgroups (Table I). Healthy controls (n = 11) were uninfected with tuberculosis as demonstrated by negative purified protein derivative (PPD) skin test and no history of immunocompromise or immunosuppressive medications. Subjects with a positive PPD >10 mm (n = 33) but with normal chest radiographs and without signs of active disease (cough, fever, weight loss) were considered to have latent tuberculosis infection. Typically, these patients belonged to high-risk groups, such as recent immigrants or injection drug users, and would be offered isoniazid preventive therapy. Finally, patients with active tuberculosis (n = 22) were defined by the presence of acid-fast bacilli in expectorated sputum and response to anti-tuberculosis therapy as documented by sputum culture conversion. After obtaining informed consent, 50 ml blood or a standard blood bank donation was collected.

**Statistical methods**

T cell responses among the three groups of subjects were analyzed in Stata IC 11.0 (StataCorp) using a nonparametric test for trend with the assumption that degree of infection is lowest in healthy controls, intermediate during latent infection, and highest during active disease. Continuous variables stratified by subgroups were analyzed by nonparametric Wilcoxon rank sums or Kruskal–Wallis tests. Categorical variables were analyzed using the Fisher’s exact test. Linear regression between two continuous variables was performed in Prism 5.0 (GraphPad Software).

**Results**

To screen for cell wall–associated substances that stimulate human T cells, we first washed intact *M. tuberculosis* bacilli with PBS to remove secreted proteins and then sterilized wet bacterial pellets by extraction with organic solvents. Organic soluble fractions were pooled to produce “cell wall extract” while the organic insoluble fraction, which was enriched for proteins, was designated “cell wall skeletons” (Fig. 1A). We tested the ability of *M. tuberculosis* cell wall extract to stimulate a T cell clone specific for ESAT-6, but observed no response, confirming the exclusion of secreted protein Ags by washing cells and extracting substances with organic solvent (Fig. 1B). By contrast, the glycolipid-specific T cell clone LDN5 was easily stimulated by cell wall extract but not by ESAT-6 or *M. tuberculosis* culture supernatants (Fig. 1C and data not shown). Thus, *M. tuberculosis* cell wall lipid Ags targeted by human T cells can be effectively separated from secreted protein Ags, and neither preparation has broadly active mitogenic effects for T cells.

We then compared T cell responses to cell wall extract and secreted Ags ESAT-6 and CFP-10 in a cohort of healthy subjects and subjects infected with *M. tuberculosis* (Table I). We considered the degree of infection to be greatest in those with active disease, intermediate in those with latent disease, and negative in healthy controls. As expected, healthy controls had the fewest IFN-γ spot-forming units (SFU) in response to ESAT-6 and CFP-10.
FIGURE 1. Generating and assessing the stimulatory capacity of cell wall extracts. (A) Scheme for generating cell wall extract by treating bacterial pellets with organic solvents chloroform (CHCl₃) and methanol (CH₃OH). (B and C) T cell clones (B) F5 or (C) LDN5 were coincubated with DCs and titrating concentrations of either cell wall extract or whole ESAT-6 protein. Data are representative of three independent experiments.

10. Greater responses among subjects with latent tuberculosis confirmed their clinical assignment on the basis of PPD test result. Notably, patients with active tuberculosis had the greatest responses, and we noted a statistically significant increase in IFN-γ SFU with degree of infection (Fig. 2A; p < 0.001). Similarly, we found that healthy controls had the fewest IFN-γ SFU in response to cell wall extract, and these also increased with degree of infection (Fig. 2B; p = 0.001). When we compared responses to cell wall extract with ESAT-6 and CFP-10, we found no difference in magnitude of response among any of the three groups of study subjects (Fig. 2C–E). Even though we had shown that cell wall extracts were not likely contaminated with ESAT-6 (Fig. 1B), we considered the possibility that the two sets of responses involved Ags that partitioned into both fractions. Importantly, we noted that many individuals responded at high levels to only one or the other Ag preparation. As a group, there was a strong correlation between responses to cell wall extract and ESAT-6 and CFP-10 (Fig. 2F; r² = 0.495, p = 0.001). Thus, cell wall Ags provide a strong stimulus to the human immune system that is distinct from the ESAT-6 protein. Data are representative of three independent experiments.

To characterize more directly its biochemical properties, cell wall extract was passed over an open silica column and serially extracted with chloroform, acetone, and methanol to separate stimulatory factors with low, intermediate, or high polarity, respectively. Fractions were dried, sonicated into media, and tested for stimulation of IFN-γ release from PBMCs. Only the methanol eluate retained stimulatory capacity, indicating that the stimulatory factor, although extractable in chloroform and methanol, was relatively polar (Fig. 4A). Our prior work revealed that methanol extracts are normally enriched in lipopeptides, so we attempted to reduce the biologic activity of methanol eluates by protease and lipase digestion (17). However, these experiments were inconclusive because antigenicity was reduced with heat treatment alone, and this step is required for inactivation of enzymes after digestion (data not shown). We also attempted direct identification of Ags using TLC and mass spectrometry, but biochemical separation by normal phase chromatography failed to resolve the biological activity into any single fraction (data not shown). Methanol eluates containing partially purified polar lipids were then used in subsequent experiments.

Because methanol eluates of cell wall extracts contain lipids, including known CD1 Ags like diodeoxymycobactins and phosphomycoketides, we hypothesized that the dominant Ags in this fraction would be CD1 restricted. Human DCs express five CD1 proteins of which four (CD1a, CD1b, CD1c, and CD1d) present unknown Ags and one (CD1e) presents CD1b Ags (18). CD1d has no known Ags. To test this hypothesis, we ligated K562 cells expressing CD1d and tested the ability of cell wall extract and ESAT-6 to stimulate IFN-γ release from PBMCs. Only ESAT-6 stimulated IFN-γ release from PBMCs, not the cell wall extract (data not shown). To determine if the lack of stimulation was due to the absence of CD1 Ags in the cell wall extract, we fractionated the cell wall extract into non-T cells, CD4+ or CD8+ and double-negative pools (Fig. 3B). Each cell type was tested for the ability to produce IFN-γ alone, and this step is required for inactivation of enzymes after digestion (data not shown). We also attempted direct identification of Ags using TLC and mass spectrometry, but biochemical separation by normal phase chromatography failed to resolve the biological activity into any single fraction (data not shown). Methanol eluates containing partially purified polar lipids were then used in subsequent experiments.

Table I. Patient characteristics of study cohort

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy Controls</th>
<th>Latent TB</th>
<th>Active TB</th>
<th>p Value</th>
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<td>11</td>
<td>33</td>
<td>22</td>
<td>—</td>
</tr>
<tr>
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<td>37 (28–48)</td>
<td>47 (38–57)</td>
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<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
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<td>17 (0–243)</td>
<td>27 (7–195)</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Statistical significance was calculated using Fisher’s exact test (sex and foreign born) or Kruskal–Wallis test (age and treatment).
with CD1a, CD1b, CD1c, or CD1d. Surprisingly, we were unable to detect T cell activation in response to cells expressing any of the four CD1 proteins (Fig. 4B). Failure to stimulate T cells was not likely due to an intrinsic defect in K562 cells because each of these transfectants has been shown to efficiently present exogenous lipid Ags and efficiently activate T cells (19). Nevertheless, we could directly test CD1 proteins in a different system in which the APC was proved to be sufficient for activation and then attempt to block the response using Abs that recognize and inhibit the function of CD1a, CD1b, and CD1d. This experiment showed that anti-CD1 did not block activation, confirming results using transfected cells. Surprisingly, anti–MHC class II, but not an isotype matched control Ab, blocked polyclonal T cell activation nearly to baseline (Fig. 4C). Taken together, these data show that organic soluble cell wall factors stimulate polyclonal T cells in an MHC class II–dependent rather than CD1-dependent manner.

MHC class II Ag loading canonically occurs via the endosomal processing pathway but can also occur at the cell surface. To distinguish between these two possibilities, we performed a T cell stimulation assay in the continuous presence of chloroquine, which inhibits endosomal acidification and has been shown to reduce MHC class II Ag processing and presentation (20). As expected, we found no effect on the capacity of DCs to present ESAT-6 peptides, which do not require cellular processing and are likely loaded at the cell surface (Fig. 4D). This result also revealed that the continuous presence of chloroquine did not globally impair DC function or lead to nonspecific T cell activation. In contrast, the stimulatory capacity of methanol eluates was markedly reduced by chloroquine treatment of DCs indicating that stimulatory factors in methanol eluates require endosomal processing.

We found that T cells were activated by an Ag-presenting molecule that binds peptides, yet the Ags were extracted by solvents commonly used for lipids. To reconcile these apparently contradictory observations, we hypothesized that MHC class II–restricted hydrophobic Ags included lipoproteins. Supporting this hypothesis, MHC class II epitopes have been identified from at least four M. tuberculosis genes annotated as lipoproteins (www.immunepitope.org). Also, a prior study implicated mycobacterial lipopeptides as T cell Ags using biochemical criteria (21). Bacterial lipoprotein synthesis is mediated by the consecutive activity of three enzymes—prolipoprotein diacylglycerol transferase (Lgt), prolipoprotein signal peptidease II (Lsp), and lipoprotein N-acyl transferase (Lnt)—resulting in triacylation of a conserved and essential N-terminal cysteine residue (Fig. 5A). Mycobacteria
produce a number of terminally acylated lipoproteins, which might have explained our prior inability to isolate stimulatory factors into a single chromatographic fraction. Rather than testing bacteria deficient in any one lipoprotein, we reasoned that deletion of the mycobacterial signal peptidase \(lspA\) would represent a reliable method to eliminate this entire class of candidate T cell Ags genetically. We generated cell wall extracts from wild-type \(M.\) \(tuberculosis\) strain H37Rv bacteria that were deficient in \(lspA\) (\(M.\) \(tb\) \(D\) \(lspA\)) and bacteria that had \(lspA\) complemented (\(M.\) \(tb\) \(D\) \(lspA::lspA\)) (16). TLC analysis of cell wall extract showed no broad differences in the migration of bands corresponding to the most abundant lipids, indicating that there was not a global change in lipid content among the bacterial mutants (Fig. 5B). This method also allowed us to normalize the abundance of total lipids derived from the three bacteria. There was minimal effect of \(lspA\) deletion on stimulation of the glycolipid-specific and CD1b-restricted T cell clone LDN5, which recognizes glucose monomycolate, a glycolipid that is not expected to be altered by \(lspA\) deletion (Fig. 5C). However, the capacity of \(M.\) \(tb\) \(D\) \(lspA\) extracts to stimulate polyclonal T cells was markedly diminished compared with wild-type, and genetic complementation of \(lspA\) restored the antigenic activity (Fig. 5D). Because \(lspA\) is required for the production of mature lipoproteins, these data indicate that lipoproteins are the source of immunodominant MHC class II–mediated stimuli in \(M.\) \(tuberculosis\) cell wall extracts.

Lipoproteins present in cell wall extracts stimulate T cells when processed and presented by DCs. However, lipoproteins are also known agonists of TLR2, so we considered the possibility of secondary stimulation of T cells after TLR2 engagement. Cell wall extracts contain a mixture of compounds including CD1-restricted glycolipids and phospholipids as well as TLR2 agonist glycolipids (22). We attempted to reduce the stimulatory effect of cell wall extract...
extracts by using an Ab that directly binds to and inhibits the function of TLR2. Preincubation of DCs with TLR2 blocking Ab did not abrogate the T cell stimulatory effect of methanol eluates (Supplemental Fig. 1). Despite the presence of multiple TLR2 agonist ligands in M. tuberculosis cell wall extract, such as phosphatidyl-myo-inositol mannosides and triacylated lipoproteins, these data reveal that T cell stimulation occurs independently of TLR2.

Discussion

In summary, our data reveal two key findings regarding human immunity to M. tuberculosis. First, using a system that emphasizes natural infection and human DCs and T cells, we identified cell wall–associated immunostimulatory factors that are distinct from the secreted Ags currently the focus of vaccine and immunodiagnostic development. Second, mechanistic analysis of the response shows that this response is dependent on DCs, involves CD4+ T cells, and is dependent on MHC class II via endosomal processing pathways as well as bacterial lspA function. Though detailed studies regarding the breadth of ex vivo T cell responses to cell wall–associated Ags are limited, our data reveal that such studies are feasible in a way that captures the immunologic and clinical complexity of human tuberculosis.

Our findings confirm and extend a previous study that used biochemical criteria to identify mycobacterial lipopeptides as Ags for cytotoxic CD4+ lymphocytes (21). Although we show that polyclonal T cell responses to cell wall Ags are strongly correlated with secreted Ags, the antigenic activity of cell wall extracts is likely due to a number of lipoproteins, whereas we compared this with overlapping peptide pools of just two proteins, ESAT-6 and CFP-10. Thus, the contribution of any single immunodominant lipoprotein remains to be determined. Little is known about the breadth of human T cell responses to mycobacterial lipoproteins. Sutcliffe and Harrington (23) identified 99 lipoproteins in M. tuberculosis by bioinformatics analysis, representing 2.5% of the proteome. The vast majority of these are pending biochemical validation, and systematic T cell epitope mapping has not been reported.

Our data show that lspA function is required for T cell activation. Because lspA catalyzes the release of the signal peptide from nascent prolipoproteins, polyclonal T cells may be recognizing signal peptide sequences as dominant Ags. Lipoproteins are characterized by the presence of a conserved “lipobox” motif (LV/ASTV/ GASC/C) on the C-terminal portion of the signal peptide (24). The N terminus of the signal peptide is less conserved and varies in length from 16 to 33 aa (23). Although this is an appropriate range for an MHC class II–restricted peptide Ag, it is less likely that such peptides would partition into organic solvents and survive solid-phase extraction in our system. Another possibility is that the failure to cleave the signal peptide results in an inability of Lnt to catalyze the final N-acylation reaction (Fig. 4A). Therefore, partially acylated lipoproteins may fail to insert into the cell wall and extract into organic solvents. Supporting this hypothesis, lspA-deficient M. tuberculosis bacilli are more susceptible to killing by malachite green and show decreased virulence, possibly as a result of increased cell wall permeability (16, 25–27). Even if partially acylated lipoproteins extract into organic solvents, they may fail to engage the proper Ag-processing pathway. Diacylated lipoproteins bind the TLR2/TLR6 heterodimer, whereas triacylated lipoproteins bind the TLR1/TLR2 heterodimer. Previous studies have shown that the 19-kDa mycobacterial lipoprotein inhibits MHC class II Ag processing via a TLR2-mediated mechanism (28). However, we show that MHC class II–dependent T cell stimulation occurs despite TLR2 blocking (Supplemental Fig. 1), suggesting lipoproteins are heterogeneous in their effects on Ag processing. Further, it is unlikely that TLR activation is a primary or specific mechanism of the CD4+ T cell activation occurring in this study because many TLR2 agonist phospholipids are present in lspA mutants, and we failed to block the response with anti-TLR2 Ab. The fact that lipoproteins are a natural combination of adjuvant and Ag may be one reason for the observed immunodominance in our polyclonal system. Recently, synthetic lipoproteins have been used successfully as vaccine immunogens in the absence of additional adjuvant (29–31). Further, vaccination with a lipid-modified epitope of the 16-kDa Ag from M. tuberculosis was shown to protect mice against aerosol challenge to a greater extent than peptide vaccination alone (32). Our results invite a reconsideration of whether such stimulatory molecules operate through the TCR, TLRs, or both.

A significant body of literature exists to support the importance of M. tuberculosis–secreted protein Ags, and some have recently suggested that the strength of T cell responses to these Ags may paradoxically assist the bacteria in its life cycle (33). T cell responses to lipids and lipoproteins are common at the polyclonal level and provide another “axis” by which we can assess the natural history of infection in humans.

Acknowledgments

We thank the patients and staff at the Lemuel Shattuck Hospital for participation in this study. We would also like to thank Aji Lalvani for recombinant ESAT-6 and CFP-10 peptide pools, John Annand for generating bacterial lipid extracts, Annemieke de Jong for providing K562 transfectants, William Jacobs for providing ΔlspA mutant and complemented bacteria, and Gwendolyn Swarbrick for logistical support.

Disclosures

The authors have no financial conflicts of interest.

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