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Research Article

Mycobacterial lipoarabinomannans modulate cytokine production in human T helper cells by interfering with raft/microdomain signalling

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Abstract. Lipoarabinomannans (LAMs) are major lipoglycans of the mycobacterial envelope and constitute immunodominant epitopes of mycobacteria. In this paper, we show that mannose-capped (ManLAM) and non-mannose-capped (PILAM) mycobacterial lipoglycans insert into T helper cell rafts without apparent binding to known receptors. T helper cells modified by the insertion of PILAM responded to CD3 cross-linking by decreasing type 1 (IL-2 and IFN-γ) and increasing type 2 (IL-4 and IL-5) cytokine production. Modification by the mannose-capped ManLAMs had similar, but more limited effects on T helper cell cytokine production. When incorporated into isolated rafts, PILAMs modulated membrane-associated kinases in a dose-dependent manner, inducing increased phosphorylation of Src kinases and Cbp/PAG in Th1 rafts, while decreasing phosphorylation of the same proteins in Th2 rafts. Mycobacterial lipoglycans thus modify the signalling machineries of rafts/microdomains in T helper cells, a modification of the membrane organization that eventually leads to an overall enhancement of type 2 and inhibition of type 1 cytokine production.

Key words. Th1/Th2; raft; signalling; cytokine; lipoarabinomannan.

The lipoarabinomannan (LAM) lipoglycans span the mycobacterial envelope [1], and are thought to insert into the mycobacterial plasma membrane by way of their glycosylphosphatidylinositol (GPI) anchor [2]. LAMs are immunodominant epitopes of mycobacteria [3, 4] that accumulate in the infected host and induce strong antibody responses [5]. Mannose capping of the LAM arabinan polymers (ManLAM) was originally considered to occur mostly in mycobacterial strains of high virulence [1], but this view was later revised when LAMs from *Mycobacterium tuberculosis* were all found to be mannose-capped, in both virulent and less virulent strains [6, 7]. Mannose-

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capped LAMs were less efficient than the non-mannose-capped LAMs (PILAMs) in modulating the cytokine production of macrophages [6–10], but recent studies have documented potent biological activities of ManLAMs [11, 12], which may be active in different contexts than PILAMs. The non-mannose-capped PILAM molecules are actually capped with a phospho-myo-inositol and should no longer be named AraLAMs, the term AraLAM being only appropriate for the rare and fully non-capped LAMs elaborated by *M. chelonae* [13].

Several surface receptors have been identified with the capacity to bind LAMs. In particular, mannose receptors on phagocytes bind ManLAMs, but not AraLAMs lacking terminal di-mannosyl units [14, 15]. Likewise, the dendritic cell surface receptor DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin) specifically recognizes capped LAMs [16]. The GPI-anchored CD14 pattern recognition receptor allows phagocytes to sense bacterial products in their environment, and binds several lipoglycans including LAMs [17] and peptidoglycans [18]. Likewise, distinct Toll-like receptors function as pattern-recognition receptors and mediate macrophage responses to LPS-CD14 or PILAM-CD14 complexes [19]. Lastly, the CD1 surface molecules accommodate ManLAMs in their large hydrophobic binding groove [20, 21] and induce immune responses [3, 4]. The ManLAM molecule can be sampled by different group I CD1 receptors in endosomes of mycobacteria-laden, antigen-presenting cells [22], and presented to a variety of CD1-restricted T cells [23].

T lymphocytes are devoid of such LAM-binding receptors, but are nonetheless functionally modified following interaction with LAMs [8, 24, 25]. In particular, Jurkat cells preincubated with LAMs downregulate mRNA levels for interleukin IL-2, IL-3 and granulocyte/macrophage-colony-stimulating factor (GM-CSF) when mitogen-activated [26], but neither the direct effects of LAMs on T lymphocytes with polarized cytokine production patterns, nor the interference of LAMs with the raft signalling platform, have been investigated.

Through differential cytokine release, T lymphocytes induce cell-mediated or humoral responses to pathogens. Among CD4 T cells, type 1 helper (Th1) lymphocytes synthesize the IL-2 and IFN-γ cytokines generally associated with resistance to intracellular infection, and favour cell-mediated immune responses such as delayed-type hypersensitivity and activation of cytotoxic T cells and macrophages, while the Th2 lymphocytes predominantly release IL-4 and IL-5 to promote B cell activation and humoral responses [27, 28]. The spectrum of leprosy skin lesions is typically polarized and ranges from tuberculoid type 1 to lepromatous type 2 responses. Patients with tuberculoid lesions present with a localized form of the disease and a strong cell-mediated response. The lepromatous leprosy patients present with multibacillary lesions and high titers of specific antibodies but poor cell-mediated immune responses against *M. leprae* antigens. Furthermore, the detection of high levels of IL-2 and IFN-γ mRNA in the total RNA extracted from lesions of tuberculoid leprosy patients contrasts with the high levels of IL-4 and IL-5 measured in lepromatous leprosy lesions [29, 30].

To further define the interaction of *M. leprae* lipoglycans with helper T cells, we investigated the effects of LAMs on the raft signalling platform and the polarized patterns of cytokine production of helper T cells. We show that LAMs insert into sphingolipid-rich rafts/microdomains of the plasma membrane of polarized T helper cells and modulate raft-associated protein kinases in a dose-dependent manner. To document the functional consequences of such events, we have analysed the modifications in the cytokine profiles of activated T helper cells after incubation with AraLAMs or ManLAMs. Our results show that LAMs inserted in T helper lymphocyte rafts alter patterns of cytokine production, most likely by modifying the transbilayer organization of the raft signalling platform.

Materials and methods

Mycobacterial lipoglycans

LAMs from a rapidly growing unclassified *Mycobacterium* species (PILAMs) and a virulent strain (Erdman) of *M. tuberculosis* (ManLAMs) were isolated as previously described [1], and found free of contaminating mycobacterial products. LAMs were obtained in freeze-dried form, reconstituted in sterile water and stored at –20 °C. Deacylated PILAM was prepared by mild alkaline hydrolysis [31]. One hundred micrograms of PILAMs was incubated in 100 µl of 0.1 N NaOH for 2 h at 37 °C, neutralized with acetic acid and desalted through Bio-Gel P-10 (Bio-Rad, Hercules, Calif.) in PBS. The eluted, deacylated PILAM was lyophilized and reconstituted in PBS. An aliquot of PILAM was processed similarly but incubated with water instead of NaOH. Lipopolysaccharide (LPS) and polymyxin B were obtained from Sigma (Fluka, Buchs, Switzerland).

Antibodies

The anti-CD3 OKT3 monoclonal antibody (mAb)-producing hybridoma was from ATCC (Bethesda, Md.). For dot-blotting, the anti-CD3ε and anti-CD4 mAbs were from Dako (DakoCytomation, Zug, Switzerland), CD55 was detected with the IA-10 mAb (BD Pharmingen, Basel, Switzerland), CD59 with MEM-43 mAb and CD45 with the MEM-28 mAb. The GM1 ganglioside was detected with the peroxidase-labelled B subunit of cholera toxin (CTB; Sigma). LAMs (AraLAM and ManLAM) were detected with the anti-LAM mAb CS35. All MEM mAbs were kindly donated by Dr. V. Horejsi, Academy of Sci-

ences of the Czech Republic, Prague. For Western blotting, the anti-Lck and anti-Fyn polyclonal anti-peptide antibodies were from Santa Cruz (LabForce, Nunningen, Switzerland) and Cbp/PAG was detected with the MEM-255 mAb.

Generation of Th1 and Th2 cell clones

Th1 and Th2 cell clones were generated from peripheral blood of normal individuals upon antigen activation and cloning by limiting dilution in RPMI-1640 medium supplemented with IL-2 (20 U/ml), penicillin (50 U/ml), streptomycin (50 µg/ml), 5% human AB serum, 10% FCS, irradiated (3500 rad) allogeneic PBMCs, and PHA (1 µg/ml) as described elsewhere [32]. Growing cells were further expanded and characterized for their capacity to produce IFN-γ and IL-4 upon CD3 cross-linking. High IFN-γ/low IL-4 producers were defined as Th1 whereas low IFN-γ high IL-4 producers were Th2 [31]. Cultured T cells were harvested 15 days after stimulation, washed extensively and suspended in RPMI-1640 medium for further studies.

Sucrose gradient centrifugation of Th1 and Th2 cells

Th1 and Th2 (50×10^6) cells were incubated in 100 µl of serum-free medium containing 20 µg PILAMs or ManLAMs (200 µg/ml) for 30 min at 37 °C. This corresponds to 0.4 µg LAMs for 1×10^6 cells, the same ratio of LAMs to cells used to evaluate the effects of LAM on cytokine mRNA and protein production. After one wash in PBS, the cells were lysed in 1% TX-100 before equilibrium sucrose gradient centrifugation as previously described and an equal volume of each fraction (20 µl) was sampled and adsorbed on nitrocellulose using the BioRad dot-blot apparatus [33]. Raft fractions (low-density fractions 3–5) were pooled, the detergent-resistant membranes pelleted by ultracentrifugation and their contents analysed by Western blotting [34].

Evaluation of the effects of mycobacterial lipoglycans on the production of T helper cell cytokines

For cytokine mRNA levels, 4×10^6 Th1 or Th2 cells were incubated in 270 µl of serum-free RPMI medium containing PILAMs or ManLAMs (6 µg/ml) for 30 min at 37 °C. Control cells were incubated under the same conditions without mycobacterial lipoglycans. Unbound LAM was removed by washing with medium containing 10% FCS. The cells were suspended in 1 ml complete medium and stimulated or not in the presence of 10 µg/ml of OKT3 and PMA (5 ng/ml) for 6 h at 37 °C. Cytokine protein levels were determined in cell-free supernatants.

RNA extraction, cDNA preparation and amplification

Th1 or Th2 cells were pelleted and lysed in 1 ml Trizol (Life Technologies), the lysate mixed with 0.2 ml chloroform, centrifuged, and the RNA precipitated from the aqueous phase by addition of 1.5 ml isopropylalcohol (Sigma). The RNA pellet was resuspended in 25 µl DEPC-treated water and its concentration measured spectrophotometrically. Complementary DNA was synthesized from RNA (0.5 µg) using 200 U of Moloney murine leukaemia virus-reverse transcriptase (Life Technologies, Gaithersburg, Md.) in a total reaction volume of 20 µl, for 1 h at 37 °C, and used as template for amplification by PCR. The sequences of oligonucleotide primers specific for β-actin, IL-2, IL-4, IL-5, IL-10 and IFN-γ were designed as shown in table 1. All primers were used at a final concentration of 1 µM. PCR was performed in a 40 µl reaction mix containing 5 µl cDNA, 1.25 units of Taq polymerase (Life

Table 1. Cytokine primers used in this study.

Primers	Sequence	Length (bp)	Size of product (bp)
β-actin			
Primer 1	GTGGGGCGCCCCAGGCACCA	20	540
Primer 2	CTCCTTAATGTCACGCACGATTTC	24	
IL-2			
Primer 1	CAGGATGCTCACATTTAAGTTTTACA	26	91
Primer 2	CTCGAGAGGTTTGAGTTCTTCTTCTA	26	
IL-4			
Primer 1	ATGGGTCTCACCTCCCAACTG	21	462
Primer 2	TCAGCTCGAACACTTTGAATATTTCTCTCTCAT	33	
IL-5			
Primer 1	CAAACGCAGAACGTTTCAGA	20	137
Primer 2	GCAGTGCCAAGGTCTCTTTC	20	
IL-10			
Primer 1	TGGTGAAACCCCGTCTCTAC	20	163
Primer 2	CTGGAGTACAGGGGCATGAT	20	
IFN-γ			
Primer 1	TGCAGAGCCAAATTGTCTCCTTTTAC	26	299
Primer 2	TTACTGGGATGCTCTTCGACCTCGAAACAGGAT	33	

Technologies), 1.0 mM $MgCl_2$ and 0.1 mM dNTP (Promega Catalysis AG, Wallisellen, Switzerland). Semiquantitative PCR analysis was carried out for 18, 22, 26 and 30 cycles and the PCR products electrophoresed on a 2% agarose gel, visualized with ethidium bromide and the resulting documents stored and processed with ImageQuant. Amplification of β-actin mRNA was used as an internal control. β-Actin levels were normalized for all samples such that the intensities of the PCR products at 18, 22, 26 and 30 cycles in different samples were identical and linear when analysed by ImageQuant. The semiquantitative analyses shown in figure 2 utilized cDNAs obtained from Th1 and Th2 cells that were pre-treated with PILAMs or ManLAMs, and stimulated with OKT3 and PMA.

Cytokine protein measurement in culture supernatants

Production in 6-h culture supernatants of IFN-γ, IL-4 (Hoffmann-La Roche, Basel, Switzerland), IL-2 and IL-5 (R & D Systems, Minneapolis, Minn.) by Th1 or Th2 cells was assessed by ELISA. The sensitivity threshold was 25 pg/ml for all assays.

In vitro kinase assays

Raft fractions (fractions 3–5, cf. fig. 1) were obtained from sucrose gradients loaded with 50×10^6 Th1 or Th2 cells, pooled and aliquoted, ultracentrifuged and resuspended in 50 µl 10 mM Hepes pH 7.4, 156 mM NaCl, containing 0, 25 or 125 µg/ml of PILAMs. After 30 min at 37 °C, raft membranes were again ultracentrifuged, resuspended in 30 µl kinase buffer and assayed for endogenous kinases as described elsewhere [35]. The levels of phosphorylation were measured by PhosphorImager and analysed by ImageQuant.

Results

PILAM and ManLAMs incorporate into rafts of Th1 and Th2 cells

Th1 and Th2 cells incubated with PILAMs or ManLAMs were washed free of unbound LAMs and subjected to subcellular fractionation in the presence of TX-100, to isolate the detergent-resistant (or raft) fractions. Dot-blot analysis of each gradient fraction of either Th1 or Th2 cells (fig. 1) showed that GM1 detected with CTB and the GPI-linked proteins CD55 and CD59 were selectively enriched in the low-density raft fractions 3–5. In contrast, the major T cell surface protein, the transmembrane CD45, was recovered in the high-density fractions 9–11 containing the TX-100-soluble proteins. The CD3ε protein was found mostly in the soluble fractions, with 10–15% reproducibly found in the raft fractions. The CD4 coreceptor distributed predominantly in the raft fractions (60%) of both Th1 and Th2 cells. The incorporated PILAM lipoglycans were only detectable in the raft fractions 3–5 with the CS35 anti-LAM mAb and the same gradient distribution was found for ManLAM. Likewise, deacylation of PILAMs or ManLAMs (see below) resulted in more than 70% inhibition of raft association. Lastly, the overall protein and ganglioside composition of rafts was not altered quantitatively or qualitatively by LAM incorporation.

PILAM-treated cells upregulate Th2 and downregulate Th1 cytokines upon stimulation by T cell receptor agonists, while ManLAM-treated cells only upregulate Th2 cytokines.

To assess the role of LAMs in cytokine production, Th1 or Th2 cells were exposed for 30 min to PILAMs or Man-

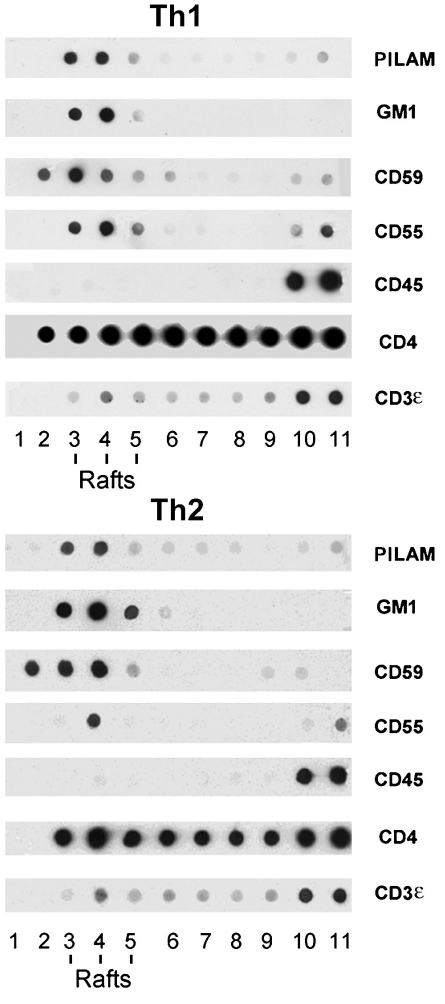

Figure 1. PILAM inserts preferentially in raft fractions of Th1 and Th2 cells. Dot-blot analysis of sucrose density gradient fractions. Each gradient was loaded with 50×10^6 Th1 or Th2 cells, previously incubated with PILAMs. Fractions 3–5 correspond to the interface between 5 and 36% sucrose (TX-100-insoluble raft membranes) and fractions 9–11 to the bulk of the TX-100-soluble proteins. Twenty microlitres was dotted for each fraction and incubated with the antibodies listed in Materials and methods, or CTB for the detection of GM1.

LAMs (0.4 µg/ml per 1×10^6 cells), leading to incorporation of lipoglycans in rafts as shown in figure 1. Under these conditions, the basal levels of cytokine mRNA were not affected. However, following activation with anti-CD3 mAb and PMA, significant changes in cytokine mRNA levels were detected by semiquantitative RT-PCR analysis in LAM-treated compared to untreated T helper cells. Exposure to PILAMs significantly decreased IL-2 and IFN-γ in Th1 cells. In contrast, IL-4 mRNA levels were increased by PILAMs, and to a lesser extent by ManLAMs, in Th2 cells (fig. 2A, B). PILAMs only increased IL-5 mRNA levels in Th2 cells (fig. 2B).

The results observed at the protein level are illustrated in figure 2C for one representative Th1 and one Th2 clone, and are comparable to the results observed at the mRNA level (fig. 2B). With all Th1 and Th2 clones tested, PILAM preincubation slightly but consistently inhibited IFN-γ and IL-2 to 80.0% ± 0.06 of the control value ($p = 0.022$, $n = 6$). Conversely, PILAMs enhanced IL-4 (149.2% ± 10.0) and IL-5 protein production (172.3% ± 3.3, $p = 0.042$, $n = 6$. ManLAM pre-incubation had no effect on IFN-γ (109.6% ± 9.8 of the control), while it enhanced IL-4 (119.8% ± 2.8) and IL-5 (192.3% ± 13.3) protein production.

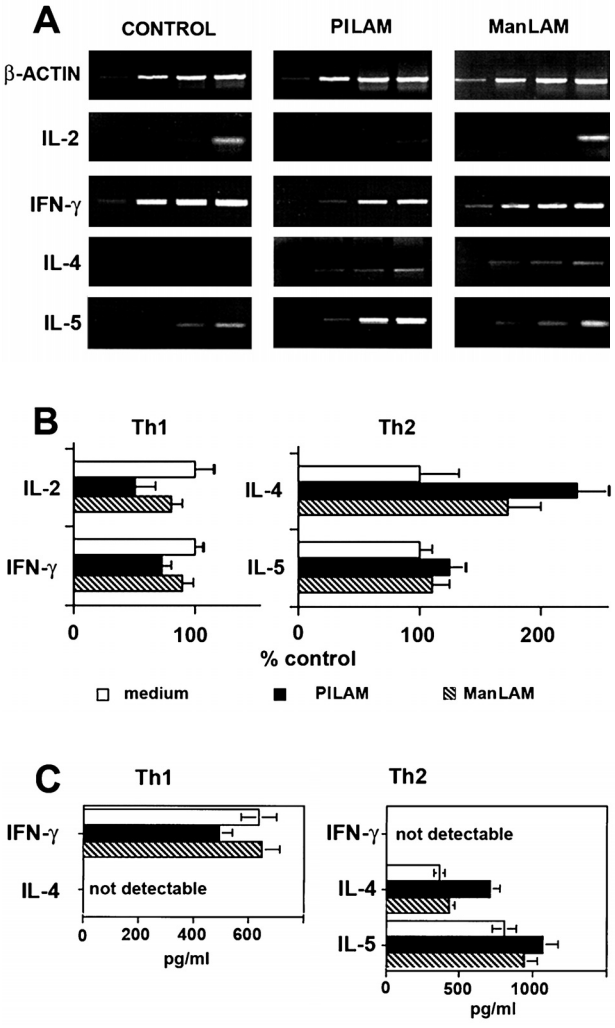

Figure 2. Effects of PILAMs and ManLAMs on cytokine mRNA and protein levels in Th1 and Th2 cells. (*A*, *B*) RT-PCR analysis of the IL-2, IFN-γ and IL-4, IL-5 responses of Th1 and Th2 cells to anti-CD3 and PMA, following pre-incubation with PILAMs or ManLAMs. Control cells were stimulated with anti-CD3 and PMA without prior incubation with mycobacterial lipoglycans. (*A*) Semiquantitative PCR for 18, 22, 26 and 30 cycles with the appropriate cytokine primers and β-actin control. (*B*) The amounts of PCR products (ethidium bromide labelled) recovered after 18, 22, 26 and 30 cycles were measured by scanning and expressed as percent of control (no LAM treatment). (*C*) ELISA measurement of the indicated cytokines in supernatants of one representative Th1 and one Th2 clone.

LAM effects are independent of LPS but depend upon the acyl moieties of the LAM molecule

Both preparations of PILAMs and ManLAMs contained 9 ng/ml of endotoxin, measured by the *Limulus* assay. The level of LPS contamination was not increased following deacylation. LPS is unlikely to have been responsible for the modulation of cytokine messages, since PILAMs and ManLAMs had different effects, despite similar contents of LPS. Moreover, upregulation of IL-4 and downregulation of IL-2 and IFN-γ by PILAMs were not affected by pre-treatment of cells with the LPS inhibitor polymyxin B.

The biological effect of PILAMs depends upon the hydrophobic part of the molecule that permits membrane insertion. Deacylated LAMs were no longer inserted into cellular membranes and thus ineffective. For instance, using Th1 cells, the inhibitory effect of PILAMs on Th1 cytokine message levels was abrogated by deacylation, while mock-treated PILAMs (i.e. subjected to the same separation steps as alkali-treated PILAMs) retained their inhibitory properties on IL-2 and IFN-γ messages (fig. 3).

Raft distribution of Lck and FynT kinases in Th1 and Th2 cells

In both Th1 and Th2 cells, the 60-kDa form of the Lck kinase was found exclusively in the raft fractions (fig. 4, top panel). The main Lck form detected in non-raft fractions

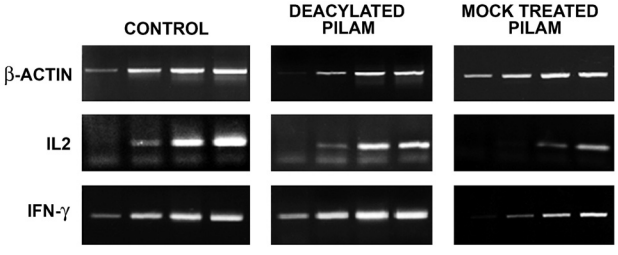

Figure 3. Deacylated PILAM fails to downregulate IL-2 and IFN-γ messages. RT-PCR of the IL-2 and IFN-γ responses of Th1 cells to anti-CD3 and PMA, with pre-incubation with deacylated PILAMs, and mock-treated PILAMs. The semiquantitative analysis of PCR products was carried out for 18, 22, 26 and 30 cycles.

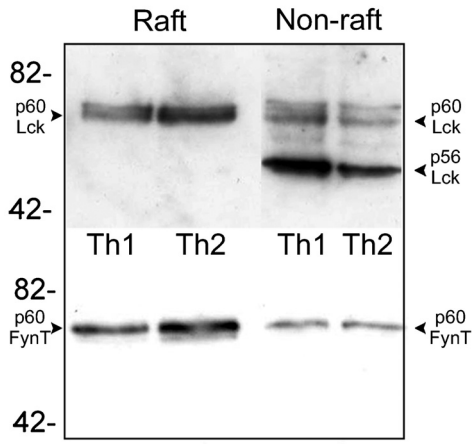

Figure 4. Raft and non-raft distribution of Lck and FynT protein tyrosine kinases in Th1 and Th2 cells. Western blot analysis. Lck (top) and FynT (bottom). Raft: pooled fractions 3–5 (20 µl); Non-raft: pooled fractions 9–11 (20 µl). The sucrose gradient was developed and fractionated as in figure 1.

was the nominal 56-kDa form. Less than 10% of the total Lck detected in non-raft fractions was 60 kDa. In both Th1 and Th2 cells, the amount of Lck found in rafts corresponded to approximately 40% of the total cellular Lck (data not shown). The FynT kinase was detected as a 60-kDa protein in both raft and non-raft fractions of both cell types with a raft/non-raft ratio of about 4 to 1 (fig. 4, bottom panel).

AraLAM incorporation in rafts modulates the in vitro activity of associated kinases

Incorporation of increasing amounts of PILAM (25 and 125 µg/ml) in Th1 raft membranes resulted in a dose-dependent increase in Src kinase (open bars) and Cbp/PAG (filled bars) phosphorylation, reaching 2.5- to 3-fold at 125 µg/ml (fig. 5A). The main phosphorylated proteins were the Src kinases (Lck and FynT) at 60 kDa and Cbp/PAG at 83 and 95 kDa. These phosphoproteins were identified by Western blotting and two-dimensional elec-

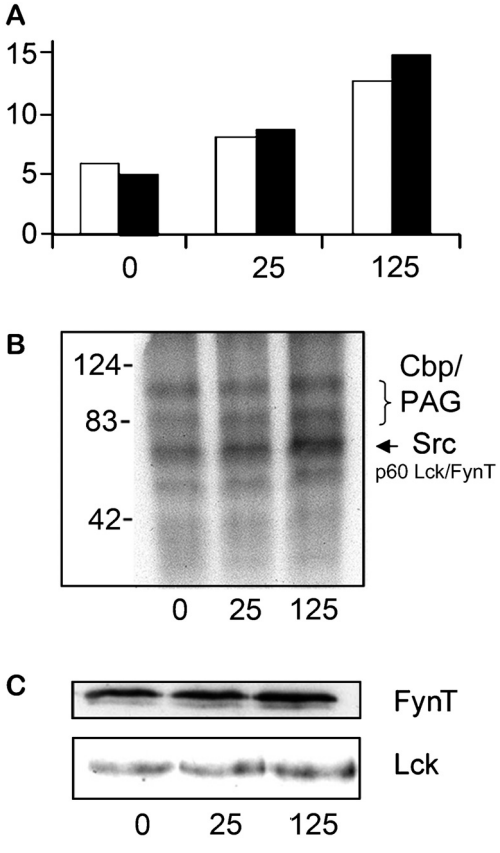

Figure 5. In vitro kinase assays on rafts isolated from Th1 cells in the presence of increasing concentrations of PILAMs (0, 25 and 125 µg/ml). (*A*) PhosphoImager measurement of Src kinase (p60Lck and FynT, open bars) and Cbp/PAG (83- and 95-kDa bands, filled bars) phosphorylation. (*B*) In vitro kinase assays following incubation of isolated rafts with 0, 25 and 125 µg/ml PILAMs. (*C*) Western blots with anti-Fyn and anti-Lck antibodies in the raft aliquots subjected to in vitro kinase assays. These results are representative of three independent experiments.

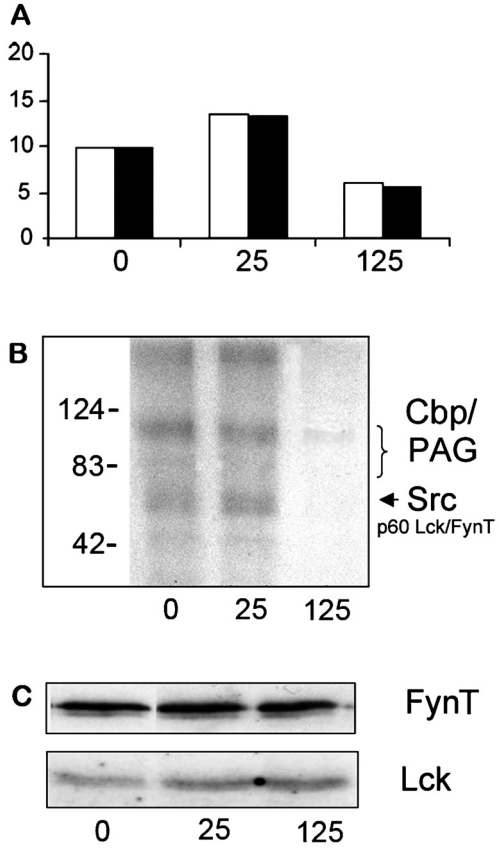

Figure 6. In vitro kinase assays on rafts isolated from Th2 cells in the presence of increasing concentrations of PILAM (0, 25 and 125 µg/ml). (*A*) PhosphoImager measurement of Src kinase (p60Lck and FynT, open bars) and Cbp/PAG (83- and 95-kDa bands, filled bars) phosphorylation. (*B*) In vitro kinase assays following incubation of isolated rafts with 0, 25 and 125 µg/ml PILAMs. (*C*) Western blots with anti-Fyn and anti-Lck antibodies in the raft aliquots subjected to in vitro kinase assays. These results are representative of three independent experiments.

trophoresis (not shown). The amounts of FynT and Lck kinases were not modified following incubation with PILAMs (fig. 5B). Incorporation of PILAMs in Th2 raft membranes under the same conditions (fig. 6A, B) caused a 25% increase in total phosphorylations with 25 µg/ml PILAMs, but all phosphorylations were downmodulated at 125 µg/ml of AraLAMs. This downmodulation was not the result of any loss of Src kinases (fig. 6C). ManLAMs caused no significant changes in the phosphorylation of Src kinases and Cbp/PAG in either Th1 or Th2 rafts (data not shown).

Discussion

This study shows that mycobacterial lipoglycans insert in raft microdomains and differentially modulate cytokine production by activated type 1 and type 2 helper T cells. Among those lipoglycans, PILAMs especially decrease mRNA levels and protein amounts of Th1 cytokines, while increasing the transcription and translation of the Th2 cytokine genes. The mannose-capped lipoglycan (ManLAM) was only efficient at increasing IL-4 and IL-5 production by Th2 cells. This is in agreement with previous reports showing that T lymphocytes respond to mycobacterial lipoglycans [24, 26], but our data show in addition that T helper cells, with GPI-linked mycobacterial LAMs inserted in their signalling platforms, respond to CD3 cross-linking by differently regulating their cytokine production.

A number of recent studies have shown a regulatory and organizing role for rafts/microdomains in signalling through the T cell receptor (TCR) [reviewed in ref. [36], as well as in cytokine signalling pathways [37, 38]. In this study, we show that insertion of mycobacterial lipoglycans in isolated rafts/microdomains alters the catalytic activity of raft-associated kinases. In particular, the autophosphorylation of Src kinases and the phosphorylation of the Cbp/PAG adaptor protein [39, 40] are modulated. The distinct dose-dependent modulations of Src kinase and Cbp/PAG phosphorylations measured in Th1 and Th2 rafts following LAM incorporation suggest a transbilayer effect of LAM on the raft signalling platform with functional modification of the raft-associated tyrosine and serine/threonine protein kinases. The different effects of LAMs on membrane-bound kinases may reflect differences in the membrane environment of FynT and Lck ratios in Th1 versus Th2 rafts and influence the functional relationships between kinases [41]. However, additional studies are needed to define which signaling pathways are affected by LAM insertion into the membrane and how the transcription factors involved in controlling Th1/Th2 cytokine production react to those proximal events.

In the Th1 and Th2 rafts analysed in this study, the form of Lck detected is reproducibly the hyperphosphorylated p60 molecule that is preferentially phosphorylated in vitro. This raft-specific form is similar to the hyperphosphorylated p60 Lck isoform expressed in activated T cells [42], but given its measurable catalytic activity, it appears distinct from the 'closed' (and catalytically less active) Lck isoform described in Jurkat cell rafts by Kabouridis [43].

The transbilayer effect of LAMs on T helper cell raft kinases is thus similar to the Lck modulation obtained with exogenous gangliosides in Jurkat T cells [44], Lyn modulation in neuronal cells [45] and protein kinase C modulation by leishmanial lipophosphoglycans in reconstituted membrane vesicles [46]. A similar transmembrane modulation by LAMs was recently reported on the Hck kinase in polymorphonuclear leucocytes [47]. In the present view of how rafts influence signalling via the TCR [48], rafts are not part of the T cell immune synapse, but rather form a concentric network around the synapse [49] and a heterogeneous population of rafts contribute distinct components to the TCR signalling machinery [50]. We propose that rafts containing GPI-anchored LAM perform differently as signalling platforms when stimulated with TCR agonists and autocrine cytokine signals. For instance, raft-associated LAMs may interact with other carbohydrate moieties on sphingolipids and glycoproteins in the context of the 'glycosynapse' on the extracellular surface of the raft [51], or inserted LAMs may modulate transmembrane receptor functions, as do gangliosides with transmembrane receptor tyrosine kinases [52].

Recent models of T cell activation envision that in the immune synapse, the TCR acts as a 'decoder' that analyzes the quality and quantity of ligand and initiates signalling, and rafts are 'amplifiers', providing the necessary adaptors and signalling proteins [53]. Our data suggest that rafts may not only amplify the response, but also qualitatively influence the T cell effector function. Indeed, we have shown that mycobacterial lipoglycans insert preferentially into sphingolipid-rich rafts of T helper cells [36] and modify the kinases associated with the raft signalling platform. Such alterations of the raft platforms probably affect both the TCR and cytokine pathways and thus alter T cell responsiveness to different stimuli. The different in vitro phosphorylation responses to LAMs measured in isolated Th1 and Th2 rafts most likely reflect the differences in the nature of signalling proteins associated with rafts [54], and further suggests that inserted LAMs may alter cellular responses by modifying the lateral and transmembrane organization of raft-associated signalling proteins without causing major changes in raft lipid and protein composition.

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