Cord factors from atypical mycobacteria \textit{(Mycobacterium alvei, Mycobacterium brumae)} stimulate the secretion of some pro-inflammatory cytokines of relevance in tuberculosis

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The ability to induce several cytokines relevant to tuberculosis (TNF-α, IL-1β, IL-6, IL-12p40 and IL-23) by cord factor (trehalose dimycolate) from \textit{Mycobacterium alvei} CR-21T and \textit{Mycobacterium brumae} CR-270T was studied in the cell lines RAW 264.7 and THP-1, and compared to the ability of cord factor from \textit{Mycobacterium tuberculosis} H37Rv, where this glycolipid appears to be implicated in the pathogenesis of tuberculosis. Details of the fine structure of these molecules were obtained by NMR and MS. The mycoloyl residues were identified as α and (ω-1)-methoxy in \textit{M. alvei} CR-21T and α in \textit{M. brumae} CR-270T; in both cases they were di-unsaturated instead of cyclopropanated as found in \textit{M. tuberculosis}. In RAW 264.7 cells, cord factors from \textit{M. alvei} CR-21T, \textit{M. brumae} CR-270T and \textit{M. tuberculosis} differed in their ability to stimulate IL-6, the higher levels corresponding to the cord factor from \textit{M. tuberculosis}. In THP-1 cells, a similar overall profile of cytokines was found for \textit{M. alvei} CR-21T and \textit{M. brumae} CR-270T, with high proportions of IL-1β and TNF-α, and different from \textit{M. tuberculosis}, where IL-6 and IL-12p40 prevailed. The data obtained indicate that cord factors from the atypical mycobacteria \textit{M. alvei} CR-21T and \textit{M. brumae} CR-270T stimulated the secretion of several pro-inflammatory cytokines, although there were some differences with those of \textit{M. tuberculosis} H37Rv. This finding seems to be due to their particular mycoloyl substituents and could be of interest when considering the potential adjuvanticity of these molecules.

INTRODUCTION

The genus \textit{Mycobacterium} includes the causative agents of tuberculosis (\textit{Mycobacterium tuberculosis}) and leprosy (\textit{Mycobacterium leprae}) together with other opportunistic or saprophytic species (atypical mycobacteria) (Falkinham, 1996). Members of this taxon present a particular cell envelope (Daffé & Draper, 1997; Brennan & Nikaido, 1995) implied in the pathogenesis of tuberculosis (Karakuosis et al., 2004). Mycolic acids (Fig. 1) constitute the characteristic lipids of the cell wall of mycobacteria; they are defined as 2-alkyl,3-hydroxy long-chain (60C–90C) fatty acids and appear mainly covalently linked to the arabinogalactan of the cell wall and also esterifying trehalose in the ‘so-called’ cord factor. This compound (Fig. 2) is a surface-displayed glycolipid identified as 6,6’-di-O-mycoloyl trehalose (Noll et al., 1956) that has attracted great interest since its discovery as a toxic substance in \textit{M. tuberculosis} (Bloch, 1950).

Cord factor takes part in some specific pathological events of tuberculosis, such as formation of granulomas (the hallmark of primary tuberculosis) and caseation (Hunter et al., 2009), participating in the upregulation of the host lipid metabolism (Kim et al., 2010). It has been demonstrated that cord factor is secreted, together with other lipids, by macrophages infected with \textit{Mycobacterium bovis} BCG (Rhoades et al., 2003) and that it is the most active lipid from a biological point of view (Geisel et al., 2005). Cord factor induces the secretion of several cytokines, both \textit{in vitro} and \textit{in vivo} (Hayashi et al., 2009; Indrigo et al., 2002, 2003; Lima et al., 2001; Welsh et al., 2008), and that of \textit{M. tuberculosis} has been employed as an adjuvant in
experimental assays of subunit vaccines against tuberculosis (Khader et al., 2007; Lima et al., 2003). In M. tuberculosis, the immuno-modulatory activity of this glycolipid has been shown to rely on the presence of cyclopropane rings and keto and methoxy groups in its mycoloyl substituents (Dao et al., 2005, 2006). Most aspects of its activity have been attributed to an insoluble crystalline conformation consisting of linear arrays of trehalose head groups alternating with exposed hydrophobic mycolic acid domains (Hunter et al., 2009). Such conformation is acquired at water–hydrophobic interfaces, including oil, polystyrene and air, and would be adopted in physiological conditions at the granuloma (Hunter et al., 2009). It is understood that the biological properties of cord factor are independent of the dose but are related to the surface area of the molecule (Hunter et al., 2009).

Studies on the biological properties of cord factor from non-tuberculous mycobacteria are scarce (Fujita et al., 2007). In the present study, the ability of cord factors from Mycobacterium alvei and Mycobacterium brumae to induce several cytokines was determined and compared with that of cord factor of M. tuberculosis H37Rv. M. alvei (Ausina et al., 1992) and M. brumae (Luquin et al., 1993) are saprophytic bacteria and it was noted since their initial descriptions that both species present a rather specific mycolic acid composition, with z- (Fig. 1a) and (ω-1)-methoxy-mycolates in M. alvei (Ausina et al., 1992; Luquin et al., 1990) and only z-mycolates (Fig. 1c) in M. brumae (Luquin et al., 1993). The structure of z-mycolates of these two species are, as a whole, similar and related to that of (ω-1)-methoxy-mycolates of M. alvei but, in contrast with M. tuberculosis (Fig. 1d,e) (Minnikin, 1982), they are diunsaturated instead of cyclopropanated. Thus, these microorganisms seemed to be good candidates for studying the possible biological activity of cord factor in atypical mycobacteria in relation to several cytokines relevant to the immunology of tuberculosis (Cooper & Khader, 2008; Russell, 2007).

**METHODS**

**Strains studied.** M. alvei CR-21 T (=ATCC 51034) and M. brumae CR-270 T (=ATCC 51384) were cultured in brain heart infusion broth at 35 °C for 1 (M. brumae) or 3 (M. alvei) weeks.

**Extraction and purification of cord factor.** Cells were recovered by filtration and lipids were extracted overnight at room temperature with, successively, chloroform/methanol at 1 : 2, 1 : 1 and 2 : 1 (all v/v). The extracts were combined, evaporated to dryness under N2 and resuspended in chloroform/methanol/water (4 : 2:1, v/v). The chloroform phase was recovered and dried, redissolved in a small volume of chloroform and precipitated (mycolic acid-containing compounds) in methanol (−30 °C, overnight, three times). The presence of cord factor was demonstrated by TLC (Mederos et al., 2010). The insoluble methanol extract was subjected to column chromatography (Mederos et al., 2010); cord-factor was recovered in the 10 % methanol fraction and finally purified by preparative TLC, employing a triple development of the plates with acetone followed by...
chloroform/methanol (9:1, v/v) (once) (Mederos et al., 2010). To discard contamination with lipopeptides/lipoproteins, purified cord factors were examined by two-dimensional TLC using the solvent systems described by Dobson et al. (1985) [first direction, chloroform/methanol/water (100:14:0.8, v/v); second direction, chloroform/acetone/methanol/water (50:60:2.5:3, v/v)] and by Eckstein et al. (2006) [first direction, chloroform/methanol (96:4, v/v); second direction, toluene/acetone (80:20, v/v)].

Cord factor from *M. tuberculosis* was purchased from Sigma and its structure was confirmed by 1H-NMR and electrospray ionization–ion trap (ESI-TRAP)-MS (see below). Additionally, its purity was confirmed by two-dimensional TLC as described above for the cord factors from *M. alvei* and *M. brumae*. This commercial glycolipid has been used by several authors (Indrigo et al., 2003; Welsh et al., 2008), and similar results to those of the cord factor purified in the laboratory of the authors have been obtained by Dao et al. (2008).

**Structural analysis of cord factor.** 1H-NMR, 1H-1H-homonuclear correlated spectroscopy (COSY)-NMR and 13C-NMR were performed at 25 °C in a 400 MHz Bruker apparatus, with cord factor dissolved (5 mg ml\(^{-1}\)) in deuterochloroform/deuteromethanol (6:1, v/v). Mycolic acids from cord factors were liberated by saponification and studied by using ESI-TRAP-MS (positive and negative modes) as described previously (Mederos et al., 2010).

**Cytokine responses to cord factor.** Cytokine responses were studied in two cell lines: RAW 264.7 (mouse leukaemia macrophage cell line, purchased from ATCC) and THP-1 (human acute monocyte leukaemia cell line, purchased from the ECCC). All materials employed were free of pyrogens (as indicated by the manufacturers). Extracts of the different cord factors were examined for endotoxin contamination employing the Limulus amebocyte lysate (LAL) test (Lonza Ibérica) following the recommendations of the manufacturer. The LAL test determines the endotoxin units (EU) ml\(^{-1}\) in the preparations; in this end-point chromogenic test, the sample (20 µl of 1 mg ml\(^{-1}\) cord factor in 2-propanol; see also Dao et al. 2008) was mixed with LAL and then with a substrate at a given dilution. The reaction was stopped and a yellow colour was developed; the absorbance was determined at 410 nm and the amount of LPS calculated from a standard curve. The absorbance value for the control purified water without endotoxin (LPS), included in the test was 0.1. All our cord factor samples were ≤0.1, and, hence, free of endotoxin.

The method described by Rao et al. (2006) was followed, with some modifications, when using the RAW 264.7 cell line (adherent cells). Cord factor was resuspended by sonication in 2-propanol (Merck) (1 mg ml\(^{-1}\)) and layered [0 µg (2-propanol alone, control), 1, 5 and 20 µg] in 24-well tissue culture plates (Nunc). RAW 264.7 cells were added (10⁶ cells) in 1 ml Dulbecco’s Modified Eagle’s Medium (high glucose), supplemented with 10% fetal calf serum, penicillin (100 U ml\(^{-1}\)) and streptomycin (100 µg ml\(^{-1}\)) and incubated at 37 °C in 5% CO₂ for 48 h. A dose of 1 µg was additionally studied at 24 and 72 h with cord factors of *M. alvei* and *M. brumae*. Supernatants were collected, centrifuged and kept at -30 °C until analysis. Levels of IL-1β and IL-6 were determined by using flow cytometry employing a cytometer Beckman Coulter FC500 and the kit FlowCytoxim multiplex (ebioscience), following the recommendations of the manufacturer. Total IL-12p40 and IL-23 were analysed using, respectively, the BDoptEIA human IL-12p40 kit (BD Bioscience) and the human IL-23 (p19/p40) platinum ELISA kit (ebioscience), following the manufacturers’ recommendations. The amounts of IL-1β, IL-6 and TNF-α were determined by means of the FlowCytomix simplex kit (humans IL-1β, IL-6 and TNF-α) (ebioscience), employing a flow cytometer Beckman Coulter FC500, according to the manufacturer’s instructions.

**Statistical analysis.** The analyses were done in triplicate and the results are expressed as mean±sD or relative percentages (THP-1 cells); general statistical differences (significance at *P*<0.05) were estimated by two-way analyses of variance (Bonferroni post tests) or Student’s t test (SPSS 15.0).

**RESULTS**

**Fine structure of cord factor**

The presence of cord factor in *M. alvei* CR-21\(^T\) and *M. brumae* CR-270\(^T\) was initially determined by TLC (not shown) and confirmed by combined NMR (Table 1) and MS (Table 2) analyses. As shown by 1H-NMR (Table 1, Figs S1 and S2, available with the online version of this paper), these molecules presented proton resonances characteristic for trehalose and mycolic acids (see Figs 1a–c and 2), as previously noted for *M. simiae* (Mederos et al., 2010). Cord factor from *M. alvei* CR-21\(^T\) (Tables 1 and 2, Fig. 2) contained α- (Fig. 1a) and (ω-1)-methoxy- (Fig. 1b) mycoloyl residues, corresponding to the whole cell composition previously reported (Luquin et al., 1990). Specific signals for (ω-1)-methoxy-substituents were detected at 1.06 p.p.m. (-CH₂ adjacent to -CH-OCH₃), see Figs 1a and 2) and 3.25 p.p.m. (-OCH₃) (see Figs 1a and 2) in the 1H-NMR spectrum (Fig. S1), and they corresponded to 36.46 p.p.m. and 55.48 p.p.m., respectively, in 13C-NMR (Table 1).
signal due to the methyne adjacent to the -OCH₃ (-CH-OCH₃, Figs 1a and 2) overlapped with deuteromethanol in ¹H-NMR (Fig. S1) and resonated at 76.98 p.p.m. in ¹³C-NMR (Table 1). *M. alvei* CR-21T contained only α-mycoloyl residues (Figs 1c and S2, Table 1). Common signals for all mycoloyl constituents of the cord factors under study included those of the -CH₃ (terminal and branched), -CH₂-, -COO, C₃H and C₂H (Figs 1a–c and 2, Table 1), and also those corresponding to cis- (5.27 p.p.m., ¹H-NMR; 129.62 p.p.m., ¹³C-NMR, see Fig. 1a–c and Table 1) and trans- (5.16 p.p.m./5.29 p.p.m., ¹H-NMR; 128.17 p.p.m./136.21 p.p.m., ¹³C-NMR, see Fig. 1a–c and Table 1) double bonds (see Figs S1 and S2 for ¹H-NMR).

According to ESI-MS-TRAP analyses (see Table 2 and Fig. S3), α-mycoloyl residues of *M. alvei* varied from

<table>
<thead>
<tr>
<th>Mycobacteria</th>
<th>Whole composition</th>
<th>Chain length/major components/ major series</th>
<th>trans/cis double bond</th>
<th>cis/trans cyclopropane</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. alvei</em> CR-21T</td>
<td>α (di-unsaturated); (ω-1)-methoxy (di-unsaturated) C₇₀–C₇₄/C₇₉–C₈₄/uneven; C₇₁–C₇₉/ C₇₆–C₈₀/even</td>
<td>0.8</td>
<td></td>
<td></td>
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<tr>
<td><em>M. brumae</em> CR-270T</td>
<td>α (di-unsaturated)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>M. tuberculosis</em> H37Rv</td>
<td>α (di-cyclopanated); methoxy (cyclopanated); keto (cyclopanated) C₇₀–C₇₄/C₈₅–C₈₉/ C₈₂–C₈₆/ C₈₃–C₈₇/ C₈₄–C₈₈/ C₈₅–C₈₉/ even</td>
<td>3.3</td>
<td>6.5</td>
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to C31, the major ones being C77 (Fig. 1a) and C79; the even series contained two trans-double bonds and the uneven series one cis- and one trans-double bond (Luquin et al., 1990) (Fig. 1a). (α-1)-Methoxy mycoloyl substituents ranged from C71 to C82, with C78 (Fig. 1b) and C76 reaching the higher levels. The even series of (α-1)-methoxy was similar in structure to the uneven series of α-mycoloyl ones, and the same was true for uneven series of both substituents. The ratio trans/cis double bonds was 0.8, so the combination of double bonds cis + cis in both α and (α-1)-methoxy should be also expected. In the cord factor of M. alvei CR-21T the ratio z/(α-1)-methoxy was 1/0.7. Mycoloyl groups of M. brumae CR-270T (Fig. S4) ranged from C68 to C79, the major ones being C76 (Fig. 1c) and C75 (Table 2). Similarly to M. alvei CR-21T the even series contained two trans-double bonds (Fig. 1c) and the uneven series one cis- and one trans-double bond (Luquin et al., 1993). The ratio of trans/cis-double bonds was 3.3.

The identity of the cord factor from M. tuberculosis was confirmed by 1H-NMR and ESI-TRAP-MS (not shown). Details of the 1H-NMR spectrum indicated the presence of α- (see Fig. 1d), keto- (see Fig. 1e) (CH3-CH-CO-, 0.98 p.p.m. and 2.45 p.p.m.) and methoxy- (see Fig. 1f) (-CH-OCH3, 2.92 p.p.m.; -OCH3, 3.27 p.p.m.) mycoloyl substituents. The ratio of α-methoxy/keto was 1 : 0.5 : 0.4 and most 2,3-disubstituted cyclopropane rings were cis (ratio cis/trans=6:5) (Table 2). Only minor (2%) cis + trans double bonds were detected. Details on the chain lengths and major components and series of mycolates from the cord factor of M. tuberculosis H37Rv are given in Table 2.

![Fig. 3. Induction of IL-6 in RAW 264.7 cells by cord factors (CF) from M. alvei CR-21T (□), M. brumae CR-270T (○) and M. tuberculosis H37Rv (●). Dose dependence was studied at 0, 1, 5 and 20 µg after 48 h of incubation. The levels of cytokine are expressed as mean ± SD of three independent experiments. Statistically significant differences are indicated (**P<0.01, see text).](image)

**Fig. 4.** Time-course induction of IL-6 in RAW 264.7 cells by 1 µg cord factor (CF) from M. alvei CR-21T (□) and M. brumae CR-270T (○) compared with control (△). The levels of cytokine are expressed as mean ± SD of three independent experiments. *P<0.01.

### Cytokine stimulation by cord factor in RAW 264.7 cells

Cord factors from M. alvei CR-21T, M. brumae CR-270T and M. tuberculosis H37Rv stimulated IL-6 (Fig. 3) in RAW 264.7 cells, but failed to induce either IL-1β or total IL-12p40 (IL12/23). Stimulation achieved by cord factor from M. tuberculosis H37Rv was higher than that of M. alvei CR-21T or M. brumae CR-270T at 5 µg or 20 µg (P<0.05). At 20 µg, M. alvei CR-21T and M. brumae CR-270T also differed in their ability to induce IL-6 (P<0.001). For cord factors (1 µg) from M. alvei CR-21T and M. brumae CR-270T, the levels of IL-6 (Fig. 4) maintained or slightly increased from 24 h to 48 h, but decreased at 72 h.

### Cytokine stimulation by cord factor in THP-1 cells

The levels of induction of the cytokines studied are shown in Fig. 5(a). In the experimental conditions employed, the cord factors from M. alvei CR-21T and M. brumae CR-270T appeared to be strong inducers of TNF-α and IL-1β; the higher stimulation of IL-6, IL-12p40 or IL-23 corresponded to the cord factor of M. tuberculosis H37Rv. Similar amounts of cord factor on coated beads were found for M. alvei CR-21T and M. tuberculosis H37Rv, and both statistically differed in the capacity of induction of the various cytokines studied: TNF-α (P=0.004), IL-1β (P<0.0005), IL-6 (P=0.034), IL-12p40 (P=0.018) and IL-23 (P<0.0005). The level of cord factor from M. brumae CR-270T on the coated beads was lower than that of the other two strains and was not included in the analysis; however, two apparently distinct patterns of induction emerged (Fig. 5b), one representing M. alvei CR-21T and M. brumae CR-270T (IL-1β + TNF-α > IL-6 + IL-12p40 + IL-23; with IL-6 + IL-12p40 < 30%) and the other representing M. tuberculosis (IL-6 + IL-12p40 > IL-1β + TNF-α; with IL-6 + IL-12p40 approximately 60%).
interest in cord factor remains open, mainly due to its adjuvant activity (Khader et al., 2007; Lima et al., 2001; Werninghaus et al., 2009) and its relation to the pathogenesis of tuberculosis (Hunter et al., 2009). Moreover, some aspects of the interaction of cord factor with antigen-presenting cells are being resolved, and recent findings have indicated that the scavenger receptors MARCO (macrophage receptor with collagenous structure) (Bowdish et al., 2009) and Mincle (a C-type lectin receptor) (Ishikawa et al., 2009; Schoenen et al., 2010) are involved in the recognition of this glycolipid. On the other hand, some molecular evidence indicates that the immunomodulatory activity of cord factor from *M. tuberculosis* relies on its mycoloyl residues (Dao et al., 2008; Rao et al., 2005, 2006) which are mostly cis-cyclopropanated (α, methoxy and keto) (Minnikin, 1982; Watanabe et al., 2001).

The cytokines investigated in this study are of interest to the immunology of tuberculosis. Briefly, IL-12p40 favours the Th1 response and the resistance to mycobacterial infections (Cooper & Khader, 2008), but the role of IL-6 is controversial. In some experiments, IL-6 appeared as a pathological cytokine, helping to maintain the granuloma (Welsh et al., 2008), and in other experiments participated in the differentiation of Th17 cells (cited by Méndez-Samperio, 2010), a subset of T cells that secrete IL-17. TNF-α is, among other functions, critical for the initiation of the granuloma (Russell, 2007), and IL-1β has a role in both innate and adaptative immunity (Korbel et al., 2008). IL-23 regulates IL-17, promoting memory cells at early infection (Khader et al., 2007), but appears to be detrimental on ongoing infection (Cruz et al., 2010). Data from the present work indicate that cord factors from *M. alvei CR-21*<sup>T</sup> and *M. brumae CR-270*<sup>T</sup>, two atypical mycobacteria, stimulated RAW 264.7 and THP-1 cells to secrete the above-mentioned cytokines. The two species differed in the fine structure of the mycoloyl substituents of the glycolipid, as expected from the composition reported for whole cells (Austria et al., 1992; Luquin et al., 1990, 1993), although they appeared, in general, to be similar in their ability to induce the secretion of the various cytokines in THP-1 cells. Cord factors from both mycobacteria actually consist of complex mixtures of numerous molecular species. It is evident that in *M. brumae* CR-270<sup>T</sup> combinations of only α-mycolates are possible, but in *M. alvei CR-21*<sup>T</sup> they are extended to α + α, α + (ω-1)-methoxy and (ω-1)-methoxy + (ω-1)-methoxy. At present, the analysis of the biological activity of cord factor from *M. tuberculosis* H37Rv has been performed with the natural substance, where six combinations of mycoloyl substituents can also be predicted.

Notably, *M. alvei CR-21*<sup>T</sup> and *M. brumae CR-270*<sup>T</sup> differed from *M. tuberculosis* H37Rv for IL-6 when both RAW 264.7 and THP-1 cells were considered; further, *M. alvei CR-21*<sup>T</sup> and *M. brumae CR-270*<sup>T</sup> differed in IL-6 induction in RAW 264.7 cells at the higher dose (20 μg) of cord factor. It seems that the structural variations noted in the mycolic acids of cord factors translated into their biological activities, but we do not have any plausible explanation for this apparent relationship. It was reported that pcaA mutants of *M. tuberculosis* (Rao et al., 2005), devoid of proximal cis-cyclopropane in α-mycolates, decreased, to some extent, the levels of IL-6, suggesting that the presence of double bonds in α-mycoloyl residues negatively affects the induction of this cytokine (Rao et al., 2005). The assays were performed with mouse bone marrow-derived macrophages, and these cells also expressed a lowered induction of TNF-α (Rao et al., 2005). By using similar approaches, oxygenated mycoloyl groups seemed to act, to some extent, as inhibitors of TNF-α (Dao et al., 2008) as mutants of *M. tuberculosis* in mmaA4 (a gene implied in the synthesis of keto- and methoxy-mycolates; see Takayama et al., 2005) increased the induction of this cytokine. These results would agree with those detected in the present study for THP-1 cells, but the research by Dao et al. (2008) also indicated that IL-12p40 is negatively controlled by oxygenated functions in the cord factor of *M. tuberculosis*. The influence of cord factor on TNF-α levels could be more
refined, because the amount of this cytokine is increased in mutants of *M. tuberculosis* lacking cmaA2 (Rao et al., 2006), which introduces a trans-cyclopropane in oxygenated mycolates (Takayama et al., 2005). Thus, results from previous studies indicate that several functional groups on mycoloyl residues of cord factor differently influence the stimulation of several cytokines, which implies that their structural peculiarities are important in the recognition mechanisms of this molecule. In macrophages and dendritic cells, Mincle appeared as an essential receptor (Ishikawa et al., 2009; Schoenen et al., 2010); nevertheless, a role for MARCO was also suggested in macrophages (Bowdish et al., 2009). MARCO acts by presenting cord factor to CD14-TLR2 (Bowdish et al., 2009), and the participation of these proteins justifies the importance of the mycoloyl groups in the recognition of cord factor and in the fine regulation of the process. It is noteworthy that MARCO is not present in RAW 264.7 cells (Bowdish et al., 2009), so the differences in the secretion profiles noted in the present work between the cited cell line and THP-1 cells can be, in part, related to this fact.

It has been reported that mycolic acids are able to stimulate by themselves the production of TNF-α, IL-6 and IL-12 in vivo (Korf et al., 2005). They also produce inflammation (Vander Beken et al., 2011), and the most potent are methoxy-mycolates containing a cis-cyclopropane ring. Keto-mycolates with the same structural features were considered anti-inflammatory, whereas ω-mycolates appeared inert (Vander Beken et al., 2011). However, our data clearly show that cord factor with only ω-mycoloyl substituents (*M. brumae*) is able to induce variable amounts of all the cytokines under study; further, mmaA4 mutants of *M. tuberculosis* (in theory containing mostly di-cis-cyclopropanated ω-mycoloyl substituents) induced high levels of TNF-α and IL-12p40 (Dao et al., 2008). Apparently, the lack of activity of ω-mycolates should rely on the transformation of macrophages to foamy macrophages (Korf et al., 2005; Peyron et al., 2008), characteristic of granulomas. Moreover, mycolic acids are recognized by a subset of T cells in a CD1-restricted way (Beckman et al., 1994) and should make an important contribution to the immunology of tuberculosis (Montamat-Sicotte et al., 2011). Thus, the interaction of mycoloyl residues of the mycobacterial cell envelope with the immune system appears to be varied and complex.

In conclusion, the pattern of induction of the different cytokines by cord factors from the atypical mycobacteria studied can be considered both similar to and, to some extent, different from that of the cord factor of *M. tuberculosis* H37Rv, probably because of the inherent variations in their mycoloyl substituents. At present, however, it is difficult to extrapolate these findings to the context of the immunology of tuberculosis but they could be of interest when considering the adjuvanticity of this glycolipid. Clearly, further analyses are required to clarify if cord factors from atypical mycobacteria could have a role as adjuvants.

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