Cellular Immunology 335 (2019) 85–92

Contents lists available at ScienceDirect

Cellular Immunology

journal homepage: www.elsevier.com/locate/ycimm

Mannosylated structures of mycobacterial lipoarabinomannans facilitate the maturation and activation of dendritic cells

Shaopeng Zhang[a], Qihang Wu[a], Hang Lei[a], Hui Zheng[a], Fang Zhou[a], Zhanqiang Sun[a], Junwei Zhao[b], Xiaoli Yu[a,*], Shulin Zhang[c,d,*]

[a] School of Biology and Pharmaceutical Engineering, Wuhan Polytechnic University, Wuhan, China
[b] Clinical Laboratory, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China
[c] Shanghai Public Health Clinical Center, Shanghai, China
[d] Department of Immunology and Microbiology, Shanghai Jiao Tong University School of Medicine, Shanghai, China

ARTICLE INFO

Keywords:
Mycobacterium tuberculosis
Lipoarabinomannan
Dendritic Cell
Cytokine

ABSTRACT

Lipoarabinomannan (LAM) is an important virulent factor secreted by mycobacteria, which generally elicit a strong immune response in the host. In this study, the structural difference of LAMs from three mycobacterial strains, *Mycobacterium tuberculosis* H37Rv, *Mycobacterium smegmatis* mc^{2}155 and a newly discovered clinical isolate, *M.* sp. QGD101, was analyzed and further evaluated whether these LAMs can induce DC maturation and promote the immunomodulatory properties. The results reveal that the major structural difference of these LAMs is the amount of mannosyl residues, especially at the terminal end of LAM, which play a key role in determining the divergent response of DCs after mycobacterial infection. Also, this study indicates an important relevance between the glycosylated structure of LAM and its immunomodulatory property, which is helpful to develop a potential approach for identification of different mycobacteria and also lays a foundation for the development of a novel polysaccharide immunological strategy against tuberculosis.

1. Introduction

Tuberculosis (TB) is one of the major causes of human death around the world. *Mycobacterium tuberculosis* (*Mtb*), the causative bacteria of tuberculosis, has developed complicated mechanisms to interfere with both the innate and acquired immune system of the host [1]. Especially at the early stage of mycobacterial infection, the interaction between mycobacteria and host immune cells is essential for *Mtb* to influence the subsequent immune responses [2].

As the most important antigen presenting cells, dendritic cells (DCs) are considered to be one of main cell populations to contact with mycobacteria after mycobacterial infection, which are mainly localized in the lung parenchyma and the epithelium of the airways [3,4]. The DCs stimulated by mycobacterial antigens can release immunoregulatory cytokines, which may induce a pro-inflammatory response and promote the activation of T cells [5].

The immune recognition is based on the detection of molecular structures that are unique to pathogens. For the mycobacterial species, various glycolipids and polysaccharide in their cell wall play an important role in the immune recognition and immune manipulation [6]. Lipoarabinomannan (LAM) is a major polysaccharide in the mycobacterial cell wall, which is also considered as an important virulence factor. The mannose-capped LAM (ManLAM) is most abundant in the pathogenic mycobacterial species, such as *M. tuberculosis* H37Rv. In addition to ManLAMs, other immunomodulatory compounds in the mycobacterial cell wall, such as phosphatidyl-inositol mannosides (PIMs) [7–9] and lipomannan (LM) [10], have also been reported to have potent inductive effects on immune cells of the host, which are rich in the non-pathogenic mycobacterial species, such as *M. smegmatis* mc^{2}155.

LAMs from different strains of mycobacteria have been shown to account for triggering distinct immune responses [11]. However, this regulatory relationship between LAM structures and LAM-induced immune responses is less studied. Especially for non-tuberculous mycobacteria (NTM), the host-LAM interaction occurring during mycobacterial infection have remained poorly characterized [12].

In this study, in order to uncover the structural distinction of LAMs from multiple types of mycobacterial strains and investigate their different immunomodulatory activities, we analyzed the structure of three types of LAMs purified from *M. tuberculosis* H37Rv, *M. smegmatis* mc^{2}155 and a clinical isolate *M.* sp. QGD101 by multiple lectin assays, and evaluated their regulatory effects on the maturation of mouse DCs

* Corresponding authors at: Department of Immunology and Microbiology, Shanghai Jiao Tong University School of Medicine, Shanghai, China (S. Zhang).
E-mail addresses: yxll268@126.com (X. Yu), shulinzhang@sjtu.edu.cn (S. Zhang).

https://doi.org/10.1016/j.cellimm.2018.11.007
Received 29 July 2018; Received in revised form 26 October 2018; Accepted 23 November 2018
Available online 30 November 2018

in vitro, and their surface receptor expression and cytokine profiles.

2. Materials and methods

2.1. Bacteria culture

A newly discovered clinical isolate *Mycobacterium* sp. QGD101, a non-tuberculous *Mycobacterium* species detected in southern-central China, had been identified and stored in CMCC (AA 2017003) by our researchers. The *M. smegmatis* mc^2155, *M. tuberculosis* H37Rv and *M.* sp. QGD101 that stored in −80 °C were firstly cultured on Löwenstein Jensen medium for 2–3 days at 37 °C. Then, H37Rv and *M.* sp. QGD101 were propagated in Middlebrook 7H9 medium for 3–4 weeks at 37 °C, and *M. smegmatis* mc^2155 was cultured for 1–2 weeks under the same condition. Large scale cultures were grown and collected in 50 ml centrifuge tubes, then were incubated at 80 °C for 2 h to inactivate mycobacterial cells. Pellets were obtained by centrifugation at 3800 × *g* for 15 min room temperature, discard the supernatant and store it at 4 °C.

2.2. LAMs extraction and purification

The crude glycolipids of LAMs were extracted by a Triton X-114 phase separation method based on early protocols [13], and purified with size exclusion columns (Sephacryl S-100) in an AKTA purifier system (Pharmacia Biotech, GE Healthcare) [14], which was efficient to separate and remove large endotoxin aggregates from LAMs. Different fractions were analyzed in SDS-PAGE (SDS-polyacrylamide gels) and glycolipid bands were then revealed with acid-silver stain.

The concentration of LAMs was respectively calculated by the phenol-sulfuric acid colorimetric method, which was performed at the wavelength of 490 nm with phenol-sulfuric acid as a color reagent.

2.3. Lectin assays

The structural differences of the purified LAMs isolated from the different strains of *Mtb* were investigated by the specific lectins (ConA, PSA, SNAI and PTLII).

The purified LAMs were separated by 15% SDS-PAGE and transferred onto polyvinylidene difluoride (PVDF) membranes. After treating with phosphate buffered saline containing 0.1% Tween-20 (PBST), membranes were respectively incubated with the corresponding biotin-lectin (5 $\mu g.ml^{-1}$) in PBST at 4 °C overnight. Three washes of 5 min each were performed with PBST. Then, membranes were incubated with 20 ml PBST, followed by an incubation with horseradish peroxidase (HRP)-avidin at a dilution of 1:500 for 1 h at room temperature [15]. Three additional washes with PBST (5 min each) were carried out on the shaking table. Finally, the labeling bands were visualized with Super Signal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific, Waltham, MA).

Four lectins were purchased from EY Laboratories (San. Mateo, CA) and Vector Laboratories (Burlingame, CA), and other chemicals were purchased from Sigma-Aldrich (Shanghai, China).

2.4. Preparation of DCs from murine bone marrow

Seven to eight-week-old female C57Bl/6 mice were purchased from the Shanghai SLAC Laboratory Animal Co. Ltd (Shanghai, China). Murine bone marrow cells (BMC) were prepared from the bone of the femur. BMCs were then seeded at 1×10^6 cells/ml in PRMI1640 medium with a combination of GM-CSF/IL-4 (10 μg/μl) in Petri dishes (Corning, Corning, NY, USA). After 4 days' culture, DCs were isolated from collected cells by positive selection using anti-CD11c MicroBeads and magnetic cell sorting (MACS; Miltenyl Biotec, Bergisch Gladbach, Germany). The surface molecules of DCs (MHCII and CD11c) were then assayed to purify these DCs to > 85% purity by flow cytometer (Becton Dickinson, Mountain View, CA, USA).

2.5. Stimulation of DCs

LAMs were first diluted to a concentration of 25 ng/μl using isopropyl alcohol. 200 μl of LAM solutions were prepared per well in 96 well plate. The culture filtrate proteins (CFPs) from *M. tuberculosis* H37Rv have been obtained by filtration through 0.22-μm polyethersulfone filters. Cells were cultured in the presence of stimulants for 6 h, 24 h and 48 h. 420 μl supernatants for each set were respectively collected and stored at −20 °C for cytokine assays.

2.6. Flow cytometry

Antibodies used for flow cytometry analysis were: anti-CD11c (IgG, clone HL3), anti-CD80 (IgG2a, clone RMMP-1) and anti-CD86 (IgG2a, clone RMMP-2) (CosmoBio, Tokyo, Japan). These cell surface molecules on DCs were assayed using a FACScan flow cytometer (Becton Dickinson, Mountain View, CA, USA). All DC maturation experiments were performed after 48 h stimulation.

2.7. ELISA assays for cytokines

Cytokines in supernatants of DC cultures (IL-1β, I-10, I-6, IL-12p40, IL-12p70, TGF-β and TNF-α) were quantified respectively by ELISA (BD Biosciences), according to the manufacturer's protocols.

2.8. Quantitative real-time PCR analysis of DCs surface receptors

To determine mRNA expression of MR (Mannose Receptor), Dectin-2 and DC-SIGN genes, DCs were collected at 6 h and 24 h after stimulation of LAMs. Total RNA from cells was isolated using RNeasy Mini Kit according to the manufacturer's protocol (Qiagen, Hilden, Germany). RNA was treated with DNase I (Invitrogen) and converted to cDNA using cDNA Reverse Transcription Kit (Applied Biosystems). mRNA expression of genes was determined by qPCR with a Power SYBR Green PCR Master Mix kit (Applied Biosystems), and relative gene expression values of all different gene targets were calculated by the $2^{-\Delta\Delta ct}$ method using *β-actin* as reference genes in data normalization.

The primer sequence was as follows: β-actin: 5′-TGGAATCCTGTGGCATCCATGAAAC-3′and 5′-TAAAACGCAGCTCAGTAACAGTCCG-3′; MR: 5′-TGCCGGGCGCTCGGACGGATG-3′and 5′-TGCTGCTTGCAGCTTGCCCTTGCCTGA-3′; Dectin-2: 5′-GGGATGCTGCCCAAATCACTGG-3′and 5′-TGGGTGATGAAATTCTGCTCCGCTTCA-3′; DC-SIGN: 5′-CCCTTGGCACTGCAGGTGCTCTTCCT-3′and 5′-GGGCAGGAGCGGCACAGTCG-3′.

2.9. Statistical analysis

All data are shown as the mean ± SD. Two-tailed *t-test* were used to assess the statistical significance. Value of $P < 0.01$ was considered as statistical significance.

3. Results

3.1. Comparison of molecular weight of LAMs

To evaluate the molecular weight of three types of LAMs, purified LAM extracts were analyzed by SDS-PAGE using 15% tricine gel by periodic acid-silver nitrate staining (Fig. 1). As shown in Fig. 1, there were no other bands except LAM contents. Two bands from *M.* sp. QGD101 and *M. smegmatis* mc^2155 were in the typical 30–35 kDa range (Fig. 1, Lane 1 and 3), another band was in the 35–40 kDa range (Fig. 1, Lane 2), indicating a weight increase of ~5 kDa for *M. tuberculosis* H37Rv.

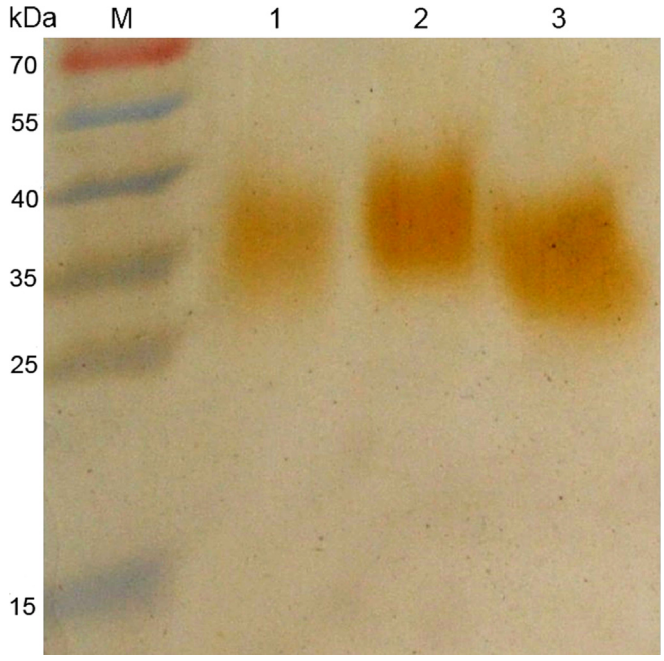

Fig. 1. SDS-PAGE profile of mycobacterial LAMs. M: Molecular weight markers. Lane 1. LAMs of *M.* sp. QGD101. Lane 2. LAMs of *M. tuberculosis* H37Rv. Lane 3. LAMs of *M. smegmatis* mc^{2}155.

3.2. Structural characterization of LAMs

Four different lectins (ConA, PSA, SNA-I and PTL-II) were used to discriminate the LAM structure by western blot detection, in which each lectin recognized specific monosaccharide based on their respective binding specificities (Table 1). After HRP labeling of lectins on a nitrocellulose membrane, two distinct bands were respectively observed towards PSA and Con A for both *M.* sp. QGD101 and *M. tuberculosis* H37Rv LAMs, but no bands were found for *M. smegmatis* mc^{2}155 (Fig. 2A). The different intensity of lectin bands between QGD101and H37Rv represented that the PSA and Con A preferentially bound to the terminal mannosyl units of H37Rv LAMs compared with QGD101, which directly showed a variation in the amount of mannose residues at the end of LAMs (Fig. 2B).

However, there were no specific reactivities for all three LAMs towards SNA I and PTL-II, which indicated that *N*-acetylneuraminic acid and *N*-acetylgalactosamine were not the constituents of the core structure of these LAMs.

3.3. Effects of LAMs on the maturation of DCs

To evaluate how DC maturation was influenced by different LAMs, the levels of surface maturation markers (CD80 and CD86) were measured by flow cytometry (Fig. 3A). After stimulation of DCs with H37Rv LAMs, the expression of CD80 and CD86 were significantly increased ($p < 0.001$) compared with levels of unstimulated cells (Fig. 3B). When *M.* sp. QGD101 LAMs were added to the DCs for 48 h, the increased expression of CD86 was also observed ($p < 0.005$) (Fig. 3B). But for *M. smegmatis* mc^{2}155 LAMs, both CD80 and CD86 were not expressed after the treatment of this avirulent strain (Fig. 3B).

Table 1
Lectins Source and Carbohydrate Specificity.

Lectin Symbol	Source	Ligand Motif
ConA	Canavalia ensiformis	Man (Terminal, Branched)
PSA	Pisum sativum	Man (*N*-acetyl chlobiose-α-Fuc)
SNA-I	Sambucus nigra	Neu5Acα2-6Gal (NAc)-R
PTL-II	Psophocarpus tetragonolobus	GalNAc, Gal

3.4. Effect of LAMs on production of DC cytokines

To assess the effect of LAM on DC function, IL-1β, IL-10, IL-6, IL-12p40, IL-12p70, TGF-β and TNF-α were detected by ELISA from DCs that were respectively cultured with LAMs, mycobacterial cells and H37Rv CFPs. All experiments were conducted with 48 h stimulation of DCs.

As expected, *M. smegmatis* mc^{2}155 LAMs displayed a significantly lower inductive activity compared with other LAMs in terms of IL-1β, IL-6, IL-12p40 and TNF-α secretion ($p < 0.01$) (Fig. 4). *M.* sp. QGD101 LAMs showed a strong and similar inductive activity as H37Rv LAMs for IL-6 production. In addition, H37Rv LAMs triggered a significant increase of IL-12p40 and TNF-α production compared with *M.* sp. QGD101 LAMs ($p < 0.01$) (Fig. 4).

And, this result also showed that DCs exposed to LAMs released fewer cytokines than DCs exposed to mycobacterial cells and CFPs, especially for the production of IL-1β, IL-6, IL-12p40 and TNF-α (Fig. 4). Furthermore, IL-10, IL-12p70 and TGF-β were almost not generated in DCs in response to LAMs stimulation. However, when LAMs were added to DCs together with CFPs, it stimulated an evident increase for cytokine outputs (Fig. 4).

3.5. Effect of LAMs on the expression of DC surface receptors

It is well known that the host immune response is initiated in response to interactions between pattern recognition receptors, such as C-type receptors, and pathogen-associated molecular patterns (PAMPs) on secreted *Mtb* antigens and cell wall components.

In this study, we measured MR, Dectin-2 and DC-SIGN expression on DCs. After stimulation for 6 h, the relative expression level of MR gene was significantly induced on DCs (Fold change > 1.5, $p < 0.01$) under LAMs treatment for both QGD101 and H37Rv (Fig. 5A). The increased expression of Dectin-2 gene was also observed (Fold change > 1, $p < 0.01$) after 6 h treatment by QGD101 LAMs (Fig. 5B). In contrast, the expression level of DC-SIGN was not up-regulated in response to 6 h post-stimulation of LAMs (Fig. 5C). Moreover, no significant transcriptional fold changes were found for all these receptors after 6 h stimulation with mc^{2}155 LAMs (Fig. 5).

After stimulation for 24 h, MR expression was significantly down-regulated under the treatment of QGD101 LAMs ($p < 0.01$) (Fig. 5A), and the expression of Dectin-2 was also obviously decreased after the stimulation of all three types of LAMs ($p < 0.01$) (Fig. 5B). In contrast, a remarkable increase in the expression of DC-SIGN gene was observed at 24 h of culture with LAMs of QGD101and H37Rv compared to the group at 6 h (Fold change > 1, $p < 0.01$) (Fig. 5C).

4. Discussion

4.1. Isolation and characterization of LAMs

LAMs are ubiquitous in mycobacteria and have been proved to modulate the host immune response [16,17]. According to the different mycobacterial species, three types of LAMs have been described: Mannose-capped LAM (ManLAM), Phospho-myo-inositol-capped LAM (PILAM) and Non-capped LAM (AraLAM) [18]. However, it is rarely to be reported for divergent immunoregulatory effects of LAMs from different species of mycobacteria.

Here, we extracted LAMs from three different species, *M. tuberculosis* H37Rv, *M. smegmatis* mc^{2}155 and clinical isolate *M.* sp. QGD101. According to the molecular weight, *M.* sp. QGD101 and *M. smegmatis* mc^{2}155 LAM were lower than that observed for *M. tuberculosis* H37Rv LAM, which indicated a general structural variation between non-tuberculous mycobacteria and tuberculous species. Based on the lectin-binding assay, we found that PSA (a mannose-binding lectin) and Con A

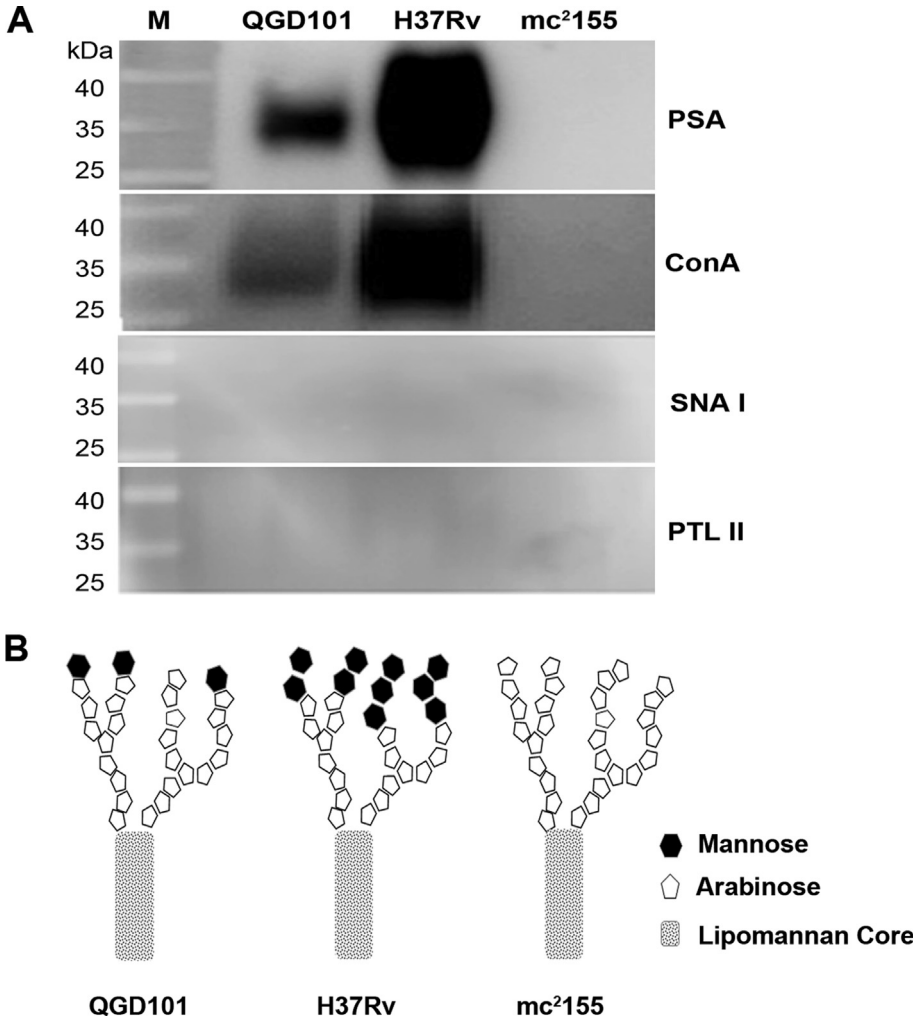

Fig. 2. Comparative analysis of the LAM structure by the lectin binding assay. (A) Western blotting of LAMs with Four lectins by the HRP labeling. M: Molecular weight markers. (B) Schematic comparison of the cap structure of LAM among H37Rv, QGD101 and mc^{2}155. For the purpose of simplicity, LAM is described as a tripartite structure composed of the mannose domain, the arabinan domain and the lipomannan (LM) core. Different mannose-cap motifs in the arabinan domain are shown.

(a terminal mannose-binding lectin) obviously bound to the LAMs of QGD101 and H37Rv compared with mc^{2}155. This was in agreement with previous findings, in which ManLAM had been described in *M. tuberculosis* H37Rv and PILAM had been found in *M. smegmatis* mc^{2}155 [18]. The difference in the lectin binding intensity between QGD101 and H37Rv demonstrated that there was a different amount of mannose units in their LAMs, especially in the cap structure of LAM (Fig. 2B). Here, we first successfully extracted and purified the LAM from *M.* sp. QGD101 that isolated from tuberculosis suspects in southern China, and preliminarily compared its LAM structure with H37Rv and mc^{2}155, which is meaningful to elaborate the connection between LAM structure and its function in the future studies.

4.2. Maturation of LAM-activated DCs

It is well known that DCs mediate interactions between innate immunity and adaptive immunity against mycobacteria [19]. Activated DCs are not only critical for the induction of primary immune responses, but also be important for the regulation of T cell–mediated immune response [20]. In the course of maturation, DCs usually express increased amounts of adhesion molecules such as CD80 and CD86 [21]. Our result showed that DCs were significantly activated with H37Rv LAMs, in terms of CD80 and CD86 expression. When compared to DCs stimulated by H37Rv, only CD86 were significantly induced by QGD101 LAMs. For *M. smegmatis* mc^{2}155 LAMs, the expression of CD80 and CD86 were not induced after stimulation. It was clear that there was an obvious difference in pathogenicity between non-tuberculous mycobacteria (*M.* sp. QGD101 and *M. smegmatis* mc^{2}155) and *M. tuberculosis* H37Rv. These data indicated that the variation of glycan pattern among these LAMs was responsible for different effects on DC maturation.

4.3. Cytokine production on DCs

The mature DCs activated by mycobacterial antigens can produce inflammatory cytokines, such as IL-6, IL-12 and TNF-α, which mediate the protective immune against mycobacterial growth in a different manner [22]. During the mycobacterial infection, IL-6 and IL-12 are necessary to induce an acquired-immunity interferon (IFN)-γ response from T cells [23]. IL-6 generally induces the generation of Th17 cells from naïve T cells, which is a key player in the immunity to extracellular bacterial infections [24]. And, IL-12p40 and IL-12p70 are two biologically active forms of IL-12 [25]. IL-12p70 is involved in the differentiation of naïve T cells into Th1 cell, which plays a vital role in the immunity to intracellular bacteria [26]. Recent evidence suggested that the IL-12p40 was more important to the generation of protective responses to mycobacterial infection than the IL-12p70 [27]. In addition, TNF-α is also an important modulator of early inflammatory

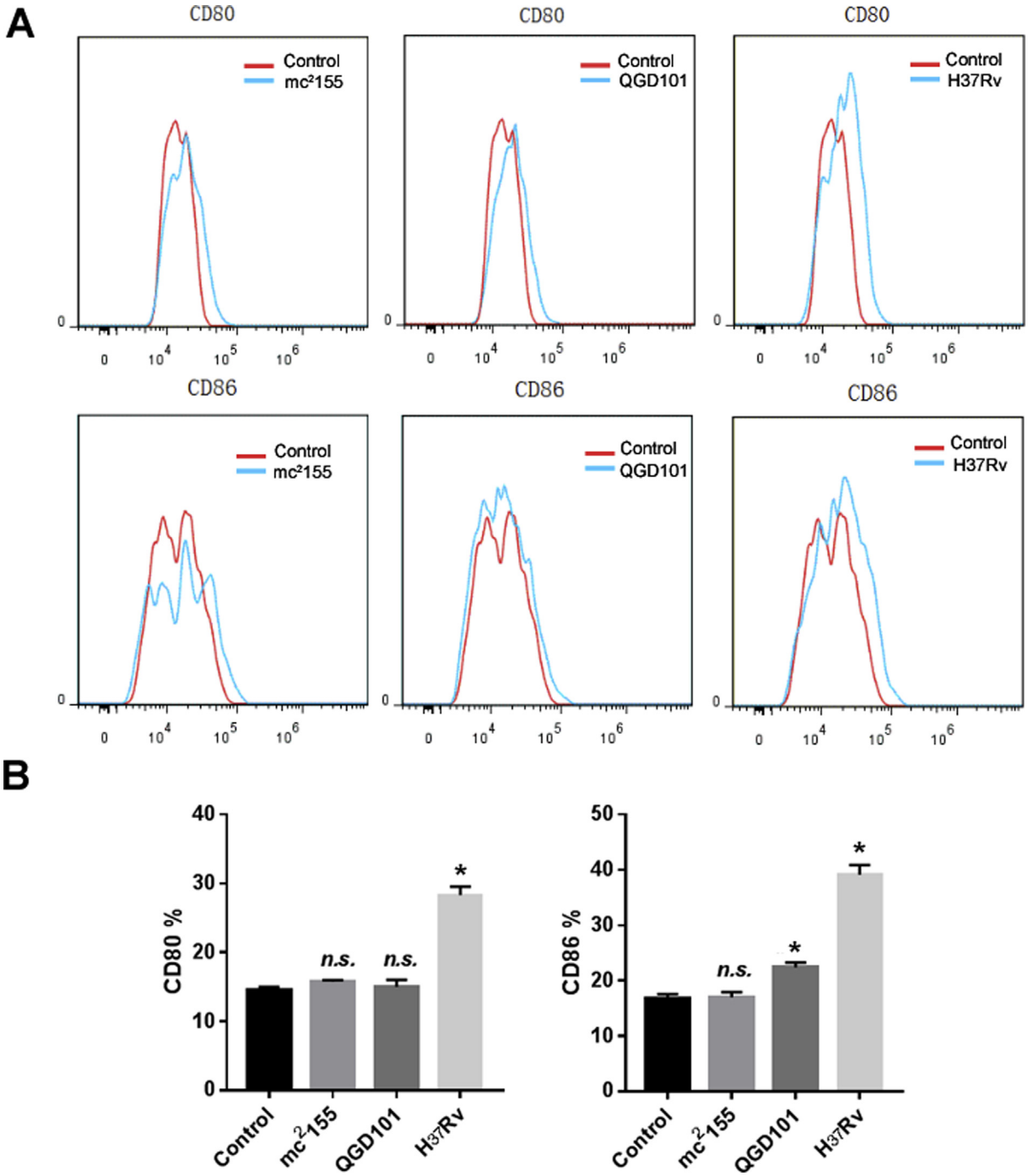

Fig. 3. The Production of CD80 and CD86 on DCs exposed to LAMs. (A) CD80 and CD86 were analyzed by flow cytometry. (B) The expression of CD80 and CD86 was compared between control and various experimental groups. The data represent means ± SD of three independent experiments. *p < 0.01 was considered to be statistically significant compared to the control column. The value of *n.s.* was defined as no significant effect.

responses to infection with *Mtb* [28]. Several studies had shown that animals in which TNF had been neutralized were more susceptible to primary TB disease after mycobacterial infection [29,30].

In this study, *M. tuberculosis* H37Rv LAMs induced a strong cytokine response in DCs, such as IL-6, IL-12p40 and TNF-α release (Fig. 4). The moderate but significant increases for IL-6, IL-12p40 and TNF-α were also observed in DCs after stimulation by *M.* sp. QGD101 LAMs (Fig. 4). Thus, it was reasonable to assume that LAMs of H37Rv and QGD101 would further promote the activation of T cell by these cytokines induced from DCs. But for *M. smegmatis* mc^{2}155 LAM, it seemed to be limited to trigger all cytokines release from DCs, which just reflected its poor ability to induce DC maturation.

As expected, three types of LAMs from *M. tuberculosis* H37Rv, *M.* sp. QGD101 and *M. smegmatis* mc^{2}155 had the different stimulatory capacity. Especially for H37Rv and QGD101 LAMs, they might elicit host immune response by promoting Th17 differentiation for defense against extracellular mycobacteria during the early stage of mycobacterial infection.

In addition, the production of cytokines was augmented when DCs were treated with LAMs together with CFPs as compared to DCs exposed to LAMs alone. Similar trends were also observed on DCs treated with mycobacterial cells. This finding suggested that different receptors

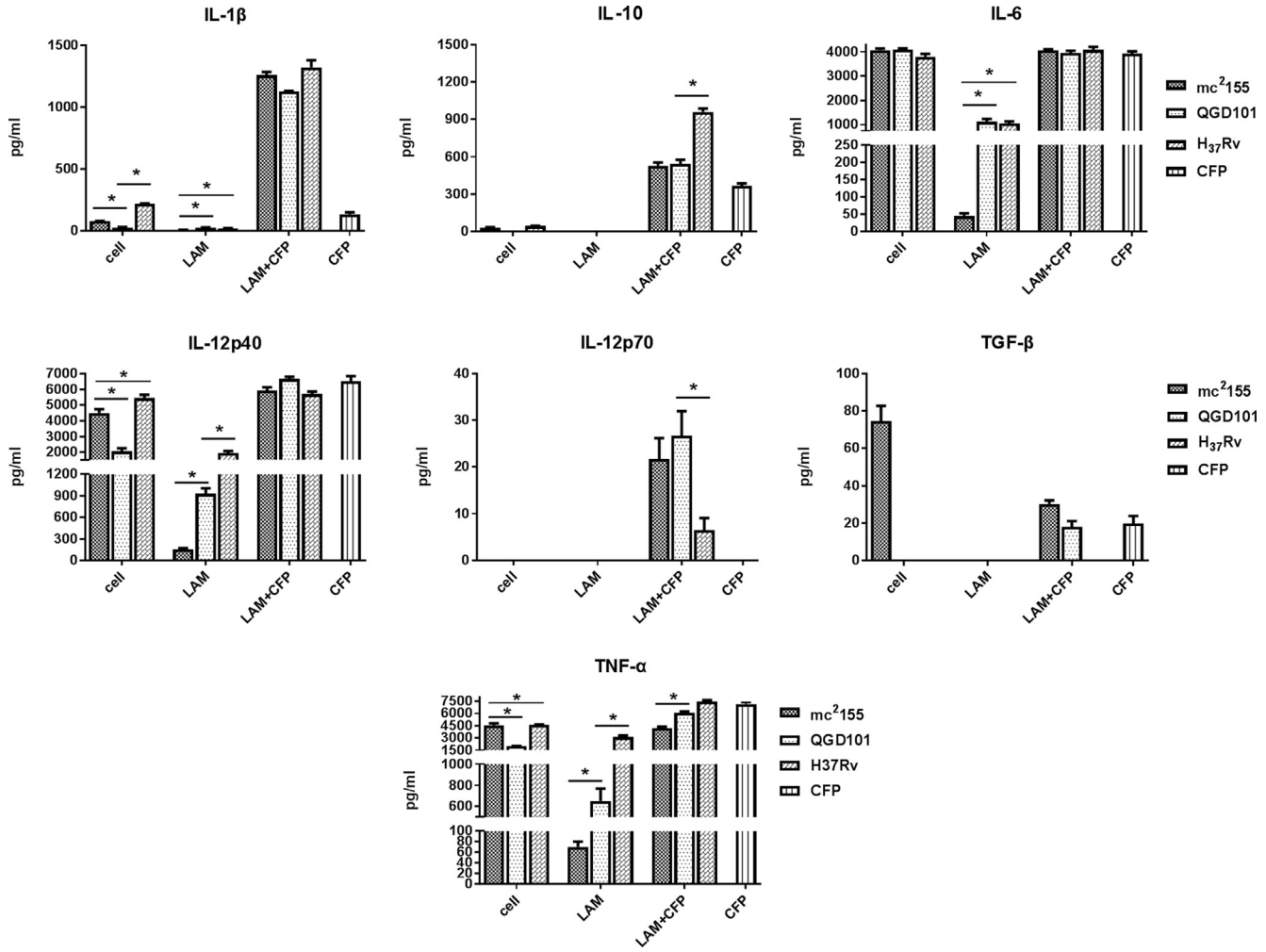

Fig. 4. Cytokines Production of DCs after 48 h for Cells, LAMs and CFPs stimulation. Secretion of IL-1β, I-10, I-6, IL-12p40, IL-12p70, TGF-β and TNF-α by dendritic cells were assayed by ELISA after 48 h exposure to mycobacterial cells, LAMs and/or CFPs. The data represent means ± SD of three independent experiments. Statistically significant differences are labeled with an asterisk (*$p < 0.01$). Horizontal lines indicate significant differences between treatment groups.

on DCs might be involved in the LAM-induced response as compared to other antigens-driven cytokine responses, such as CFP- receptor and other cell wall related receptors in mycobacterial cells.

4.4. The surface expression of DC receptors

DCs have an important function to recognize and transfer antigen information from the outside to the cells of the adaptive immune system, which relies on pattern recognition receptors (PRRs) that are expressed on the cell surface of DCs. C-type lectin receptors (CLRs) that identified as PRRs can recognize glycan structures present on the pathogens [31]. Among CLRs, there are three main receptors recognizing mannose residues exposed on pathogens or pathogenic structures, such as mannose receptor (MR), the murine dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN), and dendritic cell-associated lectin-2 (Dectin-2) [32,33]. The spectrum of carbohydrates recognized by these 'mannose-specific' lectins may be quite different. MR had eight conserved carbohydrate-recognition domains (CRDs), which had preferred specificity for terminal mannose-residues [34]. DC-SIGN exhibited broader specificity for internal mannose structures [35]. Dectin-2 exhibited weak interactions to some of the terminal mannose, which showed a lower affinity for mannose binding compared with the other two lectins [36].

Despite the apparent similarities in the mannose recognition of these receptors, they displayed a distinct response capability when different mycobacterial LAMs were used in the stimulation assay. In this study, we analyzed the transcriptional responses of MR, Dectin-2 and DC-SIGN after LAM stimulus in DCs. As expected, *M. smegmatis* mc^{2}155 LAMs exhibited no significant inductive activity for all these receptors, which represent the weak binding specificity with these receptors. In contrast, LAMs derived from *M.* sp. QGD101 and *M. tuberculosis* H37Rv significantly up-regulated the expression of MR after stimulation (Fold change > 1.5). Interestingly, these two types of LAMs significantly inhibited the expression of Dectin-2 but simultaneously induced the expression of DC-SIGN at 24 h post stimulation compared to the early stage at 6 h (Fig. 5). This indicated that DC-SIGN on DCs played a more important role in the detection of internal mannosyl moiety in LAMs during the late stage of stimulation than Dectin-2.

In summary, we report that three types of LAMs with different mannosylated structures show divergent effects on the maturation of mouse DCs and their cytokine responses, as well as triggering different expression of surface receptors on DCs. H37Rv and QGD101 LAMs that are full of capping mannose residues always induce the positive immune responses with regard to the cytokine production and receptor expression, but *M. smegmatis* mc^{2}155 LAM exhibits no significant stimulating activity. It is conceivable that the amount and scaffold of mannose in the LAM may be a crucial factor in determining the differential response of the immune system. Although our knowledge of these signaling events triggered by LAMs on DCs remains limited, we demonstrate here a direct correlation between the structural differences

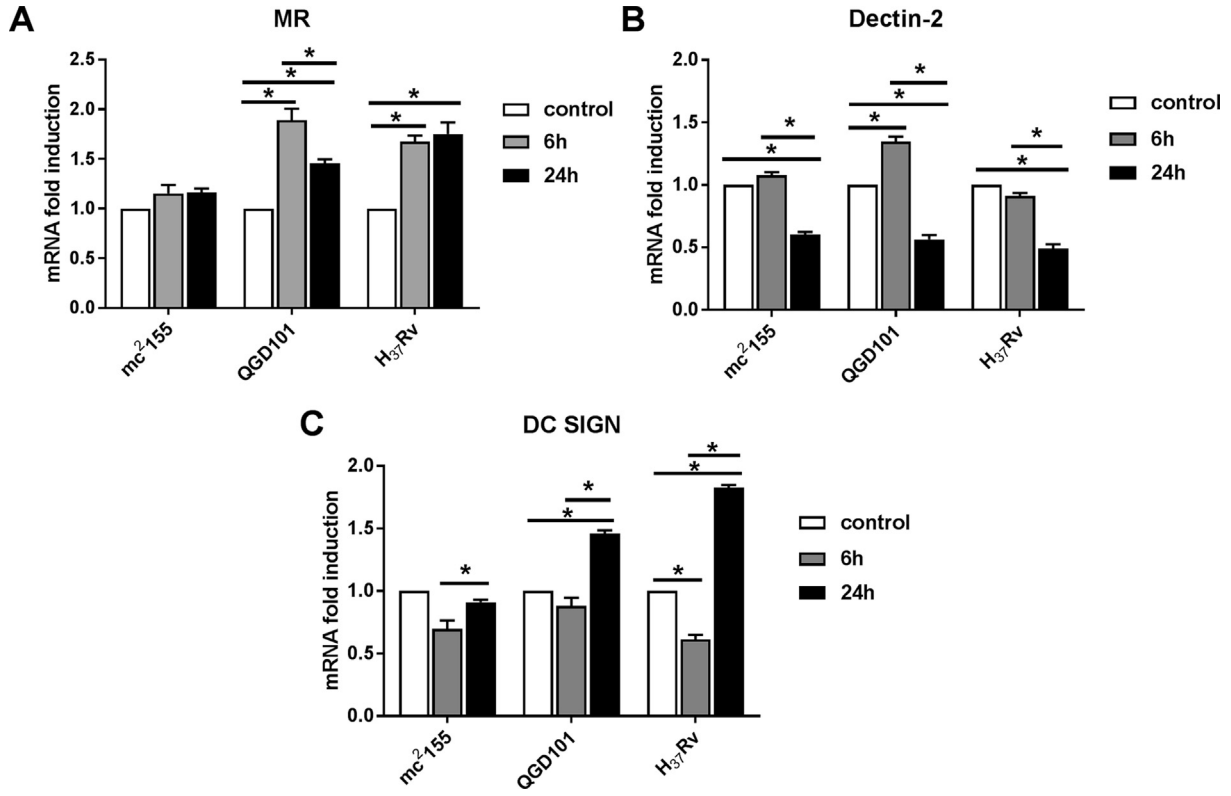

Fig. 5. Expression of MR, Dectin-2 and DC-SIGN of DC under LAMs stimulus for 6 h and 24 h. (A) MR mRNA expression. (B) Dectin-2 mRNA expression. (C) DC-SIGN mRNA expression. The bars represent the fold-change related to the control group (untreated DCs at 6 h or 24 h) which is set to 1. The data represent means ± SD of three independent experiments. Statistically significant differences are labeled with an asterisk (*$p < 0.01$). Horizontal lines indicate significant differences between treatment groups.

in mycobacterial LAMs and their immunomodulatory activities on DCs.

Acknowledgments

Financial support from the National Science and Technology Major Project (No. 2017ZX10201301-003-001, No. 2017ZX10201301-003-003), the National Natural Science Foundation of China (No. 81871613, No. 81501715) and Shanghai Jiao Tong University School of Medicine Technology Transfer Project (No. ZT201720) are gratefully acknowledged.

Conflict of interest

We would like to state that there is no conflict of interest relative to the paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cellimm.2018.11.007.

References

[1] I.M. Orme, R.T. Robinson, A.M. Cooper, The balance between protective and pathogenic immune responses in the TB-infected lung, Nat. Immunol. 16 (2014) 57.
[2] P.C. Karakousis, W.R. Bishai, S.E. Dorman, Mycobacterium tuberculosis cell envelope lipids and the host immune response, Cell. Microbiol. 6 (2004) 105–116.
[3] L. Balboa, D. Kviatcovsky, P. Schierloh, M. García, S. de la Barrera, M.D.C. Sasiain, Monocyte-derived dendritic cells early exposed to Mycobacterium tuberculosis induce an enhanced T helper 17 response and transfer mycobacterial antigens, Int. J. Med. Microbiol. 306 (2016) 541–553.
[4] A.J. Wolf, L. Desvignes, B. Linas, N. Banaiee, T. Tamura, K. Takatsu, J.D. Ernst, Initiation of the adaptive immune response to Mycobacterium tuberculosis depends on antigen production in the local lymph node, not the lungs, J. Exp. Med. 205 (2008) 105–115.
[5] A.J. Wolf, B. Linas, G.J. Trevejo-Nunez, E. Kincaid, T. Tamura, K. Takatsu, J.D. Ernst, Mycobacterium tuberculosis infects dendritic cells with high frequency and impairs their function in vivo, J. Immunol. 179 (2007) 2509–2519.
[6] L. Guenin-Mace, R. Simeone, C. Demangel, Lipids of pathogenic Mycobacteria: contributions to virulence and host immune suppression, Transbound. Emerg. Dis. 56 (2009) 255–268.
[7] B.W. Jones, T.K. Means, K.A. Heldwein, M.A. Keen, P.J. Hill, J.T. Belisle, M.J. Fenton, Different Toll-like receptor agonists induce distinct macrophage responses, J. Leukoc. Biol. 69 (2001) 1036–1044.
[8] M. Gilleron, V.F. Quesniaux, G. Puzo, Acylation state of the phosphatidylinositol hexamannosides from Mycobacterium bovis bacillus Calmette Guerin and mycobacterium tuberculosis H37Rv and its implication in Toll-like receptor response, J. Biol. Chem. 278 (2003) 29880–29889.
[9] E. Doz, S. Rose, N. Court, S. Front, V. Vasseur, S. Charron, M. Gilleron, G. Puzo, I. Fremaux, Y. Delneste, F. Erard, B. Ryffel, O.R. Martin, V.F. Quesniaux, Mycobacterial phosphatidylinositol mannosides negatively regulate host Toll-like receptor 4, MyD88-dependent proinflammatory cytokines, and TRIF-dependent co-stimulatory molecule expression, J. Biol. Chem. 284 (2009) 23187–23196.
[10] C. Vignal, Y. Guerardel, L. Kremer, M. Masson, D. Legrand, J. Mazurier, E. Elass, Lipomannans, but not lipoarabinomannans, purified from Mycobacterium chelonae and Mycobacterium kansasii induce TNF-alpha and IL-8 secretion by a CD14-toll-like receptor 2-dependent mechanism, J. Immunol. 171 (2003) 2014–2023.
[11] J. Mazurek, L. Ignatowicz, G. Kallenius, S.B. Svenson, A. Pawlowski, B. Hamasur, Divergent effects of mycobacterial cell wall glycolipids on maturation and function of human monocyte-derived dendritic cells, PLoS One 7 (2012) e42515.
[12] L.X. Heinz, K. Schroder, Novel insights into the innate immune response to non-tuberculous Mycobacteria, Immunol. Cell Biol. 90 (2012) 568–570.
[13] P. Tanya, M.R. David (Eds.), Mycobacteria Protocols, Humana Press, New York, 2015, pp. 86–96.
[14] S.H. Xuan, J.W. Zhao, Z.Q. Sun, Y.C. Zhou, C.B. Zhang, X.L. Yu, X.K. Guo, W.D. Liu, S.L. Zhang, Isolation, purification and characterization of lipoglycans from mycobacteria, Microbiol. China 41 (1) (2014) 96–103.
[15] O. Machon, S.F. Baldini, J.P. Ribeiro, A. Steenackers, A. Varrot, T. Lefebvre, A. Imberty, Recombinant fungal lectin as a new tool to investigate O-GlcNAcylation processes, Glycobiology 27 (2017) 123–128.
[16] A. Vercellone, J. Nigou, G. Puzo, Relationships between the structure and the roles of lipoarabinomannans and related glycoconjugates in tuberculosis pathogenesis, Front. Biosci. 3 (1998) e149–163.
[17] G.R. Strohmeier, M.J. Fenton, Roles of lipoarabinomannan in the pathogenesis of tuberculosis, Microbes Infect. 1 (1999) 709–717.
[18] J. Nigou, M. Gilleron, G. Puzo, Lipoarabinomannans: from structure to biosynthesis, Biochimie 85 (2003) 153–166.

[19] S. Choi, H.G. Choi, J. Lee, K.W. Shin, H.J. Kim, Mycobacterium tuberculosis protein Rv2220 induces maturation and activation of dendritic cells, Cell Immunol. 328 (2018) 70–78.
[20] H. Moll, Dendritic cells and host resistance to infection, Cell Microbiol. 5 (2003) 493–500.
[21] J. Banchereau, F. Briere, C. Caux, J. Davoust, S. Lebecque, Y.J. Liu, B. Pulendran, K. Palucka, Immunobiology of dendritic cells, Annu. Rev. Immunol. 18 (2000) 767–811.
[22] M. Moser, K.M. Murphy, Dendritic cell regulation of TH1-TH2 development, Nat. Immunol. 1 (2000) 199–205.
[23] A.M. Cooper, K.D. Mayer-Barber, A. Sher, Role of innate cytokines in mycobacterial infection, Mucosal Immunol. 4 (2011) 252.
[24] K. Akihiro, K. Tadamitsu, IL-6: regulator of Treg/Th17 balance, Eur. J. Immunol. 40 (2010) 1830–1835.
[25] P. Ling, M.K. Gately, U. Gubler, A.S. Stern, P. Lin, K. Hollfelder, C. Su, Y.C. Pan, J. Hakimi, Human IL-12 p40 homodimer binds to the IL-12 receptor but does not mediate biologic activity, J. Immunol. 154 (1995) 116–127.
[26] W.E. Paul, Th1 fate determination in CD4+ T cells: notice is served of the importance of IL-12, J. Immunol. 181 (2008) 4435–4436.
[27] A.M. Cooper, A. Kipnis, J. Turner, J. Magram, J. Ferrante, I.M. Orme, Mice lacking bioactive IL-12 can generate protective, antigen-specific cellular responses to mycobacterial infection only if the IL-12 p40 subunit is present, J. Immunol. 168 (2002) 1322–1327.
[28] J. Harris, J. Keane, How tumour necrosis factor blockers interfere with tuberculosis immunity, Clin. Exp. Immunol. 161 (2010) 1–9.
[29] J. Champsi, L.S. Young, L.E. Bermudez, Production of TNF-alpha, IL-6 and TGF-beta, and expression of receptors for TNF-alpha and IL-6, during murine Mycobacterium avium infection, Immunology 84 (1995) 549–554.
[30] A.G.D. Bean, D.R. Roach, H. Briscoe, M.P. France, H. Korner, J.D. Sedgwick, W.J. Britton, Structural deficiencies in granuloma formation in TNF gene-targeted mice underlie the heightened susceptibility to aerosol mycobacterium tuberculosis infection which is not compensated for by lymphotoxin, J. Immunol. 162 (1999) 3504–3511.
[31] A.N. Zelensky, J.E. Gready, The C-type lectin-like domain superfamily, FEBS J. 272 (2005) 6179–6217.
[32] S.J. van Vliet, J.J. Garcia-Vallejo, Y. van Kooyk, Dendritic cells and C-type lectin receptors: coupling innate to adaptive immune responses, Immunol. Cell Biol. 86 (2008) 580–587.
[33] A. Yonekawa, S. Saijo, Y. Hoshino, Y. Miyake, E. Ishikawa, M. Suzukawa, H. Inoue, M. Tanaka, M. Yoneyama, M. Oh-Hora, K. Akashi, S. Yamasaki, Dectin-2 is a direct receptor for mannose-capped lipoarabinomannan of mycobacteria, Immunity 41 (2014) 402–413.
[34] K. Drickamer, Recognition of complex carbohydrates by Ca(2+)-dependent animal lectins, Biochem. Soc. Trans. 21 (1993) 456–459.
[35] H. Feinberg, D.A. Mitchell, K. Drickamer, W.I. Weis, Structural basis for selective recognition of oligosaccharides by DC-SIGN and DC-SIGNR, Science (New York, N.Y.) 294 (2001) 2163–2166.
[36] E.P. McGreal, M. Rosas, G.D. Brown, S. Zamze, S.Y. Wong, S. Gordon, L. Martinez-Pomares, P.R. Taylor, the carbohydrate-recognition domain of Dectin-2 is a C-type lectin with specificity for high mannose, Glycobiology 16 (2006) 422–430.