Human CD4+ T cell responses have been also evaluated with recombinant *Listeria* vaccines. Guzmán and colleagues[173] noted that infection of *L. monocytogenes* expressing a helper T cell epitope (pep24) of HIV gp120 envelope glycoprotein to human macrophages and dendritic cells was capable of processing and presenting the epitope to a specific CD4+ T cell line in the context of the MHC class II molecules. These results were obtained using *hly*-deficient and *actA*-deficient carrier *L. monocytogenes* strains. The epitope was designed to be secreted, to be associated with the bacterial surface, or to be restricted to the bacterial body. Recombinant *Listeria* delivered antigens to the MHC class II pathway irrespective of *Listeria* localization in host cells (namely, phagosomes in *hly*-deficient strain and the cytoplasm in *actA*-deficient strain) or the mode of antigen display (secreted or nonsecreted).

Friedman and colleagues[174] introduced the HIV-1 *gag* gene into the highly attenuated *L. monocytogenes* Δ*dal*Δ*dat* strain (*Lmdd*). The ability of the *Listeria* (*Lmdd-gag*) to infect human monocytes and to present HIV-1 Gag antigen to CD8+ T cells of HIV-infected donors for induction of a secondary T cell immune response was evaluated. The *Lmdd-gag* provided a strong stimulus for Gag-specific CTL in HIV-infected donor peripheral blood mononuclear cells as Gag-expressing wild-type *L. monocytogenes* did. Immunization of mice with this *Lmdd-gag* strain elicited strong and prolonged CD8+ T cell responses both systematically and in Peyer's patches and mesenteric lymph nodes of immunized mice. Furthermore, Rayevskaya and colleagues[175] evaluated *Lmdd-gag* immunization in neonatal mice as well as adult mice against the challenge of vaccinia virus expressing HIV-1 Gag. Immunization of *Lmdd-gag* was protective against the challenge both in adult and neonatal mice. Upon analysis of spleen cells of mice immunized with *Lmdd*-gag, Rayevskaya and colleagues[176] found that the antigen-specific CTL activity reached a maximum at about 9 days after immunization of *Lmdd-gag*. Concomitant with the fall of CTL activity of immune spleen cells, the number of CD11a^{high} antigen-specific CD8+ T cells increased. The cells showed little or no 4-hour CTL activity, but had high delayed (18-hour) CTL activity. Zhao and colleagues[177] evaluated a modified *Lmdd* system as HIV vaccine. The authors developed a new *Lmdd-gag*, in which the transient supply of D-alanine is provided by a *dal* gene–carrying plasmid from which the *dal* gene is excised during cytosolic growth of the bacterium through the action of the *res*/resolvase recombination system.[45] Oral immunization of the recombinant attenuated *Listeria* resulted in strong dose-dependent, Gag-specific CTL in the mucosal-associated lymphoid tissues and protective immunity against vaginal challenge of HIV-1 Gag-expressing vaccinia virus.[177]

Natural transmission of HIV occurs at the mucosa and, hence, mucosa-associated lymphoid tissues may be the earliest site for virus replication. Therefore, successful induction of robust mucosal immunity may require vaccination by a mucosal route. Rayevskaya and Frankel[97] reported that parenteral immunization with *Lmdd-gag* strain provided complete protection against systemic and mucosal challenges with a recombinant vaccinia virus expressing HIV-1 Gag and that oral immunization with the attenuated strain also produced complete, long-lasting protection against the recombinant virus, but only against mucosal virus challenge. Peters and colleagues[178] showed that oral immunization of mice with HIV-1-Gag-expressing recombinant *L. monocytogenes* has led to Gag-specific responses in about 35% of lamina propria CD8+ T cells and that significant levels of Gag- and LLO-specific CD8+ T cells were observed in mucosal lymphoid tissues only after two immunizations. Immunization of recombinant *Listeria* vaccines twice enhanced T cell responses against HIV-1 Gag protein, confirming that the immunity induced against *Listeria* vector after the priming immunization does not limit immune responses induced by boosting immunization of the same *Listeria* vaccines.

To evaluate the potential of *L. monocytogenes* as a biologic vaccine vector against HIV, recombinant *L. monocytogenes* needs to be investigated in an *in vivo* challenge system. In the mouse system, a challenge study has been performed using vaccinia viruses expressing an HIV antigen (often HIV-1 Gag). Stevens and colleagues[179] used the feline immunodeficiency virus (FIV) system to evaluate recombinant *L. monocytogenes* vaccine in cats, in which FIV infection has been widespread. FIV is a pathogen of cats that induces a disease syndrome similar to that of HIV

infection in humans. Like HIV, FIV infection leads to chronic immune dysfunctions, including depletion of $CD4^+$ T cells, inversion of CD4/CD8 T cell ratios, decreased lymphocyte proliferation, and increased susceptibility to opportunistic infections. The feline model of infection and disease progression is uniquely relevant for the evaluation of vaccine design and immune response upon challenge. Cats are the natural hosts for FIV and can be infected by the vaginal route with either cell-free or cell-associated virus, thereby mimicking the natural route of infection by HIV. A single oral immunization with a novel recombinant *L. monocytogenes* strain conferred some control of viral load after vaginal challenge with FIV. Preexisting immunity to *L. monocytogenes* did not prevent induction of immune responses to FIV by a recombinant *Listeria* vaccine secreting HIV-1 Gag protein and also harboring DNA vaccine plasmid for FIV Env protein.[180]

The prime-boost vaccine strategy has been examined to improve vaccine efficacy. The combinations of naked DNA vaccination and viral vector, such as the vaccinia virus Ankara strain, have been examined.[181] The combinations of naked DNA vaccination and recombinant *Listeria* vaccination have been examined against simian immunodeficiency virus (SIV), a counterpart of HIV in nonhuman primates.[100–102] Boyer and colleagues[100] reported a prime-boost study using a nonhuman primate (rhesus monkeys) with a DNA vaccine and a recombinant *Listeria* vaccine that expresses and secretes SIV Gag and Env antigens followed by a challenge with SIV239. A recombinant DNA vaccine delivered intramuscularly and a recombinant *L. monocytogenes* delivered orally have the ability to induce $CD8^+$ and $CD4^+$ T cell immune responses in a nonhuman primate. The combined vaccine was able to induce cellular immune responses in the nonhuman primate. Thus, this strategy enhanced the efficacy of a DNA vaccine. Further, the same group reported that the DNA prime-oral *Listeria* boost strategy induced mucosal SIV-Gag-specific $CD8^+$ T cell responses characterized by expression of the $\alpha 4\beta 7$ integrin gut-homing receptor.[101]

15.5 ANTIBACTERIAL VACCINES

With over 8 million new cases and 2 million deaths each year and the appearance of multi-drug-resistant *M. tuberculosis* strains, tuberculosis still remains an urgent public health problem worldwide.[182] Since the protective efficacy of the currently used *M. bovis* BCG vaccine strain ranges from 0 to 85% in different controlled studies,[183] there is a great need for an improved vaccine. DNA vaccines have been shown to be one of the most promising new approaches to this end. Miki and colleagues[72] reported on the induction of specific protective cellular immunity against *M. tuberculosis* employing vaccination with recombinant attenuated *L. monocytogenes* strains (Table 15.4). C57BL/6 mice immunized intraperitoneally with the attenuated self-destructing *L. monocytogenes* Δ2 strain carrying plasmids for eukaryotic expression of *M. tuberculosis* Ag85 complex molecules

TABLE 15.4
Recombinant *L. monocytogenes* Vaccines for Bacteria and Parasites

Attenuation	Antigen	Promoter	Immunization Route	Immune Responses	Protection	Ref.
		Mycobacterium tuberculosis				
mpl, actA, plcB	Ag85A, Ag85B MPT51	CMV-IE (episomal)	i.v., i.p.	T cells	+	72
		Leishmania major				
actA	LACK	*hly* (episomal)	i.v.	CD4	+	193
actA	LACK	*hly* (chromosomal)	i.g., i.p.	CD4	+	192
actA	LACK	*hly* (chromosomal)	i.g.	CD4	+	194
Wild type	IL-12	*actA* (episomal)	i.p.	CD4	+	196

(Ag85A and Ag85B) and the MPB/MPT51 molecule showed specific Th1-type cellular immune responses. Furthermore, BALB/c mice immunized intravenously with these recombinant strains mounted protective cellular immunity against intravenous challenge with *M. tuberculosis* H37Rv comparable to that evoked by the conventional live *M. bovis* BCG vaccine strain.

Because *L. monocytogenes* membrane-perforating protein LLO plays a key role in activating $CD4^+$ and $CD8^+$ T cell responses, it has been applied in other bacterial systems to enhance immune response and protection. For example, the tuberculosis vaccine *M. bovis* BCG was combined with *L. monocytogenes hly* gene (encoding LLO) to form a recombinant BCG (*hly*+ rBCG), which improved specific protection against *M. tuberculosis*. By creating a urease C-deficient *hly*+ rBCG (i.e., Δ*ureC hly* + rBCG) to provide an intraphagosomal pH closer to the acidic pH optimum for *hly* activity, a much higher vaccine efficacy and cross-priming were obtained. This recombinant strain (i.e., Δ*ureC hly* + rBCG) also conferred profound protection against the *M. tuberculosis* Beijing/W genotype, for which the parental BCG is only marginally effective.[184] Recombinant *M. bovis* strains secreting *L. monocytogenes* LLO improved MHC class I presentation of cophagocytosed soluble protein, leading to enhanced capacity to stimulate $CD8^+$ T cells and protection against tuberculosis.[185]

15.6 ANTIPARASITIC VACCINES

Pathogenic parasites such as malaria parasites and *Leishmania* have their peculiar complex life cycles, which are divided into multiple stages. The appropriate types of immune responses have been required for protective immunity for these parasitic diseases. Cell-mediated immunity has been shown to be indispensable to contain intracellular protozoa such as *Leishmania*, *Toxoplasma*, and *Trypanosoma*.[186] Therefore, intracellular protozoa have been major target organisms of *Listeria*-mediated vaccines. So far, *Listeria*-mediated vaccines have been examined for the murine infection model of *Leishmania major*, an obligate intracellular protozoan (Table 15.4).

Leishmania exists in two major forms: the promastigote, which is a flagellated organism found in the sand fly, and the amastigote, which multiplies within macrophages of the host animals. Once inoculated into the mammalian host, the promastigotes rapidly invade cells such as macrophages and are transformed to amastigotes. Different from *Trypanosoma* and *Toxoplasma*, *Leishmania* can survive after the phagosomes have fused with the lysosomes. Therefore, antigens are degraded in the phagosomes and tend to be presented by the MHC class II pathway, leading to inducing specific $CD4^+$ T cells. *Leishmania*-specific $CD8^+$ T cells may also play a role in protective immunity against *Leishmania* infection, but the extent to which the T cell subset contributes remains to be examined.

Especially, Th1-type responses are required for effective protection against *L. major* infection. The murine infection model of *L. major* has been considered as one of the best models of Th1/Th2 dichotomy; the disease resistance correlates with the appearance of Th1-type immune responses to *L. major* and the susceptibility with that of Th2-type immune responses to the parasite.[187,188] IL-12 plays a central role in initiating protective immune responses against *L. major* infection by promoting IFN-γ production and the development of Th1-type immune responses.[189] A protein designated as a *Leishmania* homolog of receptors for activated C kinase (LACK) has been shown to be the major protective antigen and used as a target gene with *Listeria* carrier vaccine studies.[190] LACK is a 36-kDa protein that is highly conserved among various *Leishmania* species and expressed at both promastigote and amastigote stages.

Using naked DNA vaccine, Gurunathan and colleagues[191] showed that vaccination with DNA encoding LACK conferred protective immunity to mice infected with *L. major*. Soussi and colleagues[192] established the efficacy of an attenuated Δ*actA* recombinant *L. monocytogenes* strain expressing the heterologous LACK protein of *L. major* to induce protective Th1 $CD4^+$ T cell responses. In the study, they adopted a chromosomal integration-type plasmid system for expression of the LACK antigen, in comparison with a previous report in which an episomal plasmid system for LACK antigen expression was used.[193] The multicopy plasmid in the episomal plasmid

system was not retained for more than 48 hours and the presence of readily detectable Th1-type LACK-reactive T cells was ineffective to limit *L. major* lesion progression in BALB/c mice. In contrast, when a chromosomal integration-type plasmid system was used for the antigen expression, significant protection against *L. major* infection was observed in BALB/c mice, ranging from delay in the lesion onset to full protection in 80% of the challenged mice, depending on the size of the parasite inoculum for challenge. They also compared the intragastric route and intraperitoneal route for immunization of the attenuated *Listeria* vaccine. It appeared that the intragastric route led to a higher protection level than did the intraperitoneal one.

Saklani-Jusforgues and colleagues[194] described the local and extraintestinal dynamics of the $CD4^+$ T cell populations primed after enteric delivery of the attenuated recombinant *L. monocytogenes*. They examined the timing, magnitude, and persistence of the LACK-reactive IFN-γ- and IL-4-secreting $CD4^+$ T cell immune responses generated during enteric immunization with the *Listeria* vaccine in the Peyer's patches, mesenteric lymph nodes, spleen, liver, and blood. Efficient priming of IFN-γ-secreting LACK-specific $CD4^+$ T cells was detected in all tested tissues. These results indicate that enteral immunization of the Δ*actA L. monocytogenes* mutant efficiently induced $CD4^+$ T cells. They used intragastric delivery with an acid-adapted *L. monocytogenes* strain that survived the low gastric pH and spread physiologically along the whole gut.[195] In terms of induction of protective immunity, intragastric injections of *actA*-deficient *L. monocytogenes* expressing LACK antigen led to slower lesion progression and more chronic process without ulceration compared with the unimmunized group after a subcutaneous high-dose *L. major* challenge (2×10^5 stationary-phase promastigotes). Even though no recall responses were observed at the systemic level after intragastric reinoculations, both the delay in the onset of the lesion and the attenuation of clinical signs in the *L. major*-infected cutaneous site suggested the existence of local immune responses that extend the functions of these locally primed $CD4^+$ T cells to the skin-draining lymph nodes.

L. monocytogenes is also useful for the delivery of expression plasmids encoding immunomodulatory cytokines in addition to genes for the target antigen molecules. Shen and colleagues[196] showed that *Listeria* delivered *IL-10* and *IL-12* genes into mammalian cells, which led to regression of the progress of *Leishmania* infection. While injection of wild-type *L. monocytogenes* into hind legs of mice limited local *L. major* expansion, inoculation of IL-12-expressing *L. monocytogenes* resulted in an enhanced protective effect. It was possible that the injected *Listeria* modulated the murine immune responses to Th1-type responses, which are favorable to the inhibition of *L. major* infection. Co-delivery of genes for cytokines and antigenic proteins by using one or two separate plasmids of *L. monocytogenes* may lead to a much higher vaccine efficacy in the future.

15.7 CONCLUSIONS AND PERSPECTIVES

L. monocytogenes is an extraordinary intracellular bacterium that can be exploited as a vaccine vehicle for initiating both $CD4^+$ and $CD8^+$ T cell responses, promoting IFN-γ and IL-12 production, and delivering protective molecules into the cytoplasm. In comparison with other microbial vector systems, live *Listeria*-carrying vaccine platforms have several distinct advantages, such as mucosal route of immunization, propensity to infect professional APCs, ease of genetic manipulation, adjuvanticity, amplification of DNA vaccine plasmids *in vivo*, and simplicity of handling and storage. At present, live attenuated *L. monocytogenes* vaccines mostly belong to either auxotrophic mutants or virulence gene-deficient mutants. Auxotrophic mutants typically include a double mutant of the alanine racemase gene (*dal*) and the D-amino acid aminotransferase gene (*dat*), which mutants require D-alanine supplementation for growth, and a series of *aro* mutants such as *aroA* and *aroB* with deletions in genes of the common branch of the biosynthesis pathway leading to aromatic compounds. Virulence gene–deficient mutants frequently comprise mutants of the *hly* gene encoding LLO and *actA* gene encoding ActA. Use of these live attenuated *Listeria*-carrying vaccines has contributed to the development of enhanced protective immunity against other bacterial, viral, and parasitic infections.

Expression of genes encoding target antigens can be driven by the *Listeria* promoter or eukaryotic promoter (bactofection). A variety of episomal plasmids in *Listeria* and genome-integration plasmids (controlled by *Listeria* promoter) and episomal DNA vaccine plasmids (controlled by eukaryotic promoter) has been developed. Genome-integration plasmids enable maintenance of heterologous antigen genes in *Listeria* and hence long-time production of antigens *in situ* compared with episomal vaccine plasmids. Further, many systems have been developed for retention of plasmids episomally in *L. monocytogenes*. Target pathogens of *Listeria*-carrying vaccines reported so far contain viruses (e.g., HIV, LCMV, and influenza virus), bacteria (e.g., *M. tuberculosis*), and parasites (e.g., *L. major*). Since *Listeria*-carrying vaccines are capable of inducing specific $CD8^+$ and $CD4^+$ T cell responses and effective protective immunity, they show enormous promise for developing more effective vaccine against intracellular pathogens.

To date, the live attenuated *L. monocytogenes* strains have been derived from parent strains of high virulence and pathogenicity, which often provoke a potent immune response in the host through generations of large amounts of IFN-γ and other molecules, contributing to the host's illness. While deletion or modification of one or two key genes may reduce the pathogenicity of these strains, it may not change many other aspects of *L. monocytogenes* strains, such as the level of IFN-γ production. It is possible that these attenuated strains may maintain their ability to induce a strong immune response, which may be unnecessary and potentially detrimental to the vaccine recipients. The fact that *L. monocytogenes* consists of naturally avirulent strains (of serotype 4a) with the capability of stimulating a durable immunity against listeriosis highlights the potential benefit of using the naturally avirulent strains as vaccine carriers. Given that avirulent serotype 4a strains stimulate relatively small amounts of IFN-γ in comparison with EGD, they may cause fewer side effects without sacrificing their ability to induce an effective T cell immune response. Therefore, once the ability of recombinant naturally avirulent stains to initiate specific immunity to pathogens of interest is confirmed, it can be envisaged that naturally avirulent strains will offer a useful alternative to the live attenuated strains for delivering protective molecules against infective agents of medical and veterinary importance.

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In: Glycolipids: New Research
Editor: Daiki Sasaki, pp. 99-116

ISBN: 978-1-60456-216-3

Chapter IV

Mycobacterial Glycolipids and Host Responses

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Abstract

Mycobacterial diseases, including tuberculosis, leprosy, and disease due to nontuberculous mycobacteria, are the major cause of death from infectious diseases around the world. About one-third of the world population is latently infected with *Mycobacterium tuberculosis*. Over 8 million new cases and nearly 2 million deaths occur each year resulted from tuberculosis. Tuberculosis presents a significant health threat to the world. The pathogenicity of mycobacteria is related to their ability to escape killing by ingested macrophages, latent infection, and induce delayed-type hypersensitivity, such as granulomas. This has been attributed to several components of the mycobacterial cell wall, such as, complement activation factor, heat-shock protein, mycobacterial DNA binding protein, and glycolipids, including mycoloyl glycolipids (containing cord factor/trehalose dimycolate), phenolic glycolipid, sulfolipid, glycopeptidolipid, phosphatidylinositol mannoside, lipoarabinomannan, and lipomannan. Among them, glycolipids specific for mycobacteria play a key role in the pathogenesis, because mycobacteria are rich in lipids that possess pleiotropic activities. The complex interaction between a range of mycobacterial components and the host participate the pathogenesis. In this review we will focus on the pleiotropic activity of glycolipids in both the microbe and host. The better understanding of mycobacterial pathogenicity may open the new avenue for the development of diagnostics, therapeutic and prophylactic interventions.

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Abbreviation

M. avium-intracellulare complex, MAC;
cord factor/trehalose dimycolate, TDM;
phenolic glycolipid, PGL;
sulfolipid, SL;
glycopeptidolipid, GPL;
phosphatidylinositol mannoside, PIM;
lipoarabinomannan, LAM;
lipomannan, LM;
trehalose 6-monomycolate, TMM;
glucose-6-monomycolate, GMM;
interleukin, IL;
tumor necrosis factor, TNF;
interferon, IFN;
vascular endothelial growth factor, VEGF;
mannose-6-monomycolate, MMM;
fructose-6-monomycolate, FMM;
Toll-like receptor, TLR-2

Introduction

Robert Koch had reported that the pathogenic germ of tuberculosis was acid-fast bacteria in 1882 [1]. After that, tuberculosis has been the most serious problem as the leading cause of death from a single infectious agent in the world. About one-third of the world population is latently infected with *Mycobacterium tuberculosis*, which is one of acid-fast bacteria. Over 8 million new cases and nearly 2 million deaths occur each year resulted from tuberculosis [2]. In the members of the genus *Mycobacterium*, *M. tuberculosis*, *M. avium-intracellulare complex* (MAC), and *M. leprae* are the major pathogen in humans. The host defense against mycobacterial infection is finally intensive two aspects. One is anti-microbial defense, and the other is granulomatous inflammation that is a specific type of chronic reaction characterized by accumulations of modified macrophages (epithelioid cells). A granuloma is a focal area of granulomatous inflammation. It consists of a microscopic aggregation of macrophages that are transformed into epithelium-like cells surrounded by a collar of mononuclear leukocytes, principally lymphocytes and occasionally plasma cells. The precise mechanism for the development of disease is not fully understood.

The first step of infection is intracellular survival in the phagocytic cells, and this process is partially regulated by cell surface glycolipids as virulent factors. The most prominent feature of acid-fast bacteria is to possess a numerous kinds of lipid containing glycolipids and phospholipids in the cell envelope, and their amounts occupy 10 to 40% of the total cell, that is very high compare to other pathogenic bacteria [3]. These lipid components are considered to be influence to not only bacterial physiology and morphology but also host responses. In particularly, mycolic acid, α-branched-β-hydorxy fatty acid is a unique in nature, and

ubiquitous in *Actinomycetales* containing *Mycobacterium*, *Nocardia*, *Rhodococcus*, *Gordona*, and *Corynebacterium*. The carbon-chain length of mycolic acid is species-specific, and those of mycobacteria are the longest carbon-chain length (C70-90) [4, 5]. It is becoming evident that these lipid components are related to both host responses and bacterial pathogenesis [6-9]. Until now, many characteristic glycolipids derived from mycobacteria have been reported, for example mycoloyl glycolipids (representative cord factor/trehalose dimycolate, TDM), phenolic glycolipid (PGL), sulfolipid (SL), glycopeptidolipid (GPL), phosphatidylinositol mannoside (PIM), lipoarabinomannan (LAM), and lipomannan (LM) [10, 11]. In this review, we focus the major glycolipids of mycobacteria from the aspect of biochemistry, biology, and immunology.

Heterogeneity of Glycolipids and Phospholipids in Mycobacterial Species

The cell envelope of acid-fast bacteria, including mycobacteria, is wax-like, and rich in lipids. Proposed structure of mycobacterial cell envelope is shown in Figure 1 [10-12]. The distribution of glycolipids, but not phospholipids, is heterogeneous among mycobacterial species. The lipid is composed of the virulent (*M. tuberculosis* H37Rv and *M. tuberculosis* Aoyama B) and avirulent (*M. tuberculosis* H37Ra) strains by using two-dimensional thin-layer chromatography (Figure 2 and Table 1). The composition of glycolipids differs markedly in each strain. Some glycolipids, such as cord factor/TDM and trehalose 6-monomycolate (TMM), exist ubiquitously, but the amount was inconstant. Several glycolipids, including SLs and penta-acyl trehalose, exist only in virulent strains. In general, the composition and content of glycolipids, representative SLs, are heterogeneous among mycobacterial species.

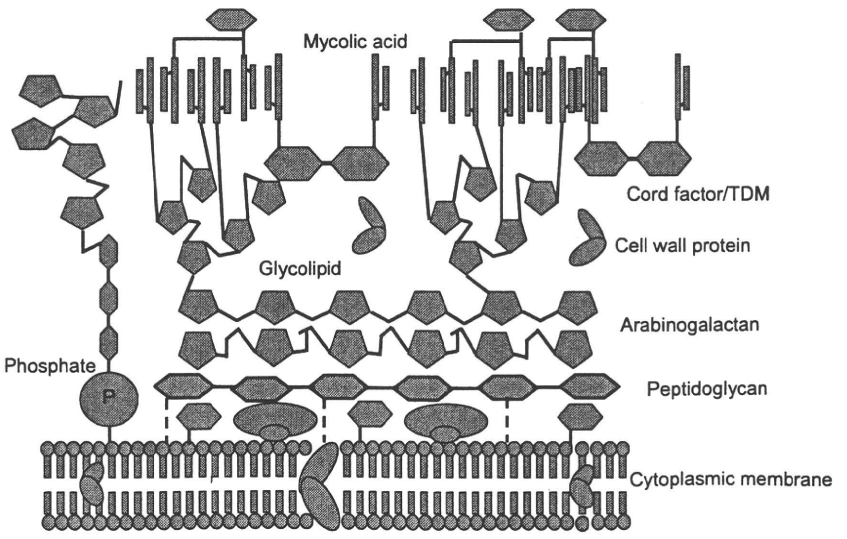

Figure 1. Schematic mycobacterial cell envelop.

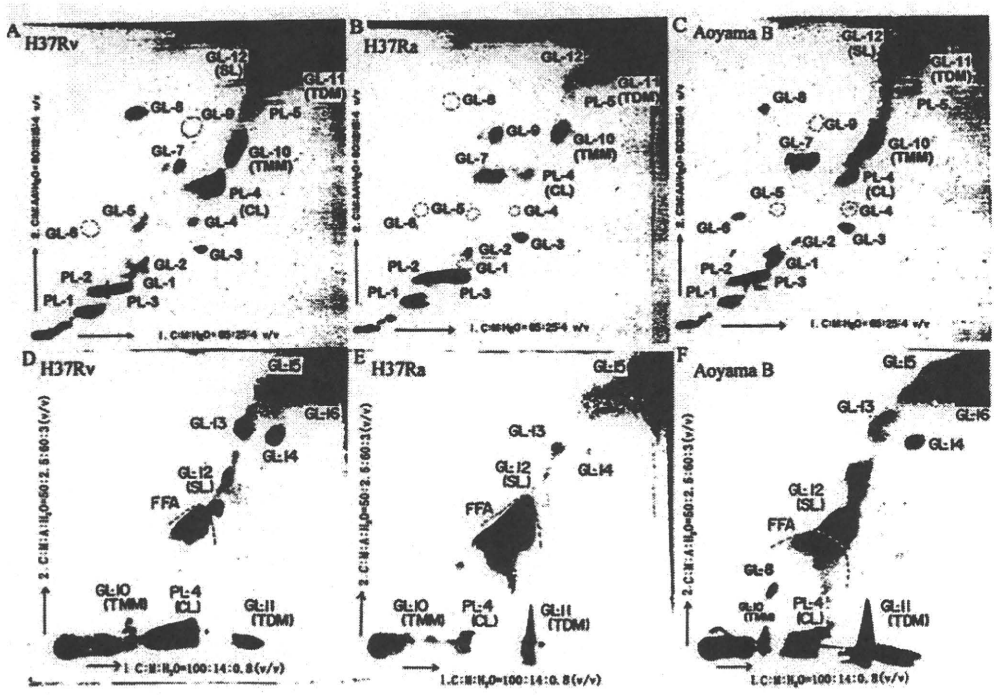

Figure 2. Two dimensional thin-layer chromatography of extractable lipid derived from virulent and avirulent mycobacterial strains. A and D, *M. tuberculosis* H37Rv; B and E, *M. tuberculosis* Aoyama B; C and F, *M. tuberculosis* H37Ra.

The Pathogenicity of Cell Wall Glycolipids

Mycobacteria are the intracellular pathogens. Mycobacterial infection results in dual consequences of the host, development of inflammatory lesions and clearing the pathogen. Genetic regulation and the expression of cell-mediated immunity and delayed-type hypersensitivity play critical roles in the outcome. Cell-mediated immunity participates in host defense, whereas delayed-type hypersensitivity involves in development of granulomatous inflammation. After mycobacterial invasion to the host cell, the bacteria multiply inside macrophages. The mechanism of pathogenesis is focused on escape from host killing system and induction of delayed-type hypersensitivity [13]. Certain cell wall glycolipids, such as cord factor/TDM, SL and LAM [14-16], involve in the host-pathogen interaction, and induce the primary host immune responses. According to current model of the mycobacterial cell envelope (Figure 1), mycolate attached to arabinogalactan forms one highly arranged leaflet of a second membrane, and other lipids participate in the construction of the outer leaflet. This structure composed a very hydrophobic barrier, and is considered to be responsible for resistance to certain drugs.

Table 1. Distribution of phospholipids and glycolipids in strains of *M. tuberculosis*

Strain	Phospholipid						Glycolipid															
	1	2	3	4	5	6	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
H37Rv	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+
Aoyama B	+	+	+	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+
H37Ra	+	+	+	+	+	+	-	+	+	-	-	-	+	-	+	+	+	-	+	+	+	-

PL-1, 2, phosphatidylinositol mannosides; PL-3, phosphatidyl ethanolamine; PL-4, diphosphatidylglycerol (cardiolipin)

GL-1, di-acyl-trehalose-2'-sulfate (SL-3); GL-8, tri-acyl-trehalose-2'-sulfate (SL-2); GL-10, trehalose-6-monomycolate (TMM); GL-11, trehalose-6,6'-dimycolate (cord factor/TDM); GL-12, penta-acyl-trehalose-2'-sulfate (SL-1); GL-16, penta-acyl-trehalose (PAT). +, present; -, absent

Table 2. Pleiotropic activities of cord factor/TDM derived from *Mycobacterium tuberculosis*

Types of granulomas	Induction of both foreign-body and hypersensitivity granulomas	
Immunogenicity	Expression of cell-mediated immunity	
Angiogenesis	Neovascularization around granulomas	
Apoptosis	Thymocytes, splenocytes	
Chemokines/Cytokines	Chemokines:	
	CXC-chemokine; IL-8	Increased
	CC-chemokines; MCP-1, MIP-1α	Increased
	Proinflammatory; IL-1β, TNF-α	Increased
	Immunoregulatory:	
	Th1-related; IL-12, IFN-γ	Increased
	Th2-related; IL-4	Depressed
	Angiogenic:	
	Vascular endothelial growth factor (VEGF), IL-8	Increased

Table 3. Structurally defined serotype-specific oligosaccharide of GPLs from *M. avium-intracellulare* complex

Serotype	Species	Haptenic oligosaccharde structure
1	*M. avium*	α-L-Rha*p*-(1→2)-L-deoxy-Tal
2	*M. avium*	4-*O*-Ac-2,3-di-*O*-Me-α-L-Fuc*p*-(1→3)-α-L-Rha*p*-(1→2)-L-deoxy-Tal
3	*M. avium*	2,3-di-*O*-Me-α-L-Fuc*p*-(1→4)-ß-D-Glc*p*A-(1→4)-2,3-di-*O*-Me-α-L-Fuc*p*-(1→3)-α-L-Rha*p*-(1→2)-L-deoxy-Tal
4	*M. avium*	4-*O*-Me-α-L-Rha*p*-(1→4)-2-*O*-Me-α-L-Fuc*p*-(1→3)-α-L-Rha*p*-(1→2)-L-deoxy-Tal
7	*M. intracellulare*	4-(2-OH)propionamido-4,6-dideoxy-2-*O*-Me-ß-Hex*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→2)-L-6-deoxy-Tal
8	*M. avium*	4,6-*O*-(1-carboxyethylidene)-3-*O*-Me-ß-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→2)-L-deoxy-Tal
9	*M. avium*	4-*O*-Ac-2,3-di-*O*-Me-α-L-Fuc*p*-(1→4)-ß-D-Glc*p*A-(1→4)-2,3-di-*O*-Me-α-L-Fuc*p*-(1→3)-α-L-Rha*p*-(1→2)-L-deoxy-Tal
12	*M. intracellulare*	4-(2-OH)propionamido-4,6-dideoxy-3-*O*-Me-ß-D-Glc*p*-(1→3)-4-*O*-Me-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→2)-L-deoxy-Tal
14	*M. intracellulare*	4-formamido-4,6-dideoxy-2-*O*-Me-3-*C*-Me-α-L-Man*p*-(1→3)-2-*O*-Me-α-D-Rha*p*-(1→3)-α-L-Rha*p*-(1→2)-L-deoxy-Tal
17	*M. intracellulare*	3-(3-OH-2-*O*-Me)butanamido-3,6-dideoxy-ß-D-Glc*p*-(1→3)-4-*O*-Me-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→2)-L-deoxy-Tal
19	*M. intracellulare*	3,4-di-*O*-Me-ß-D-Glc*p*A-(1→3)-3-*C*-Me-2,4-di-*O*-Me-α-L-Rha*p*-(1→3)-α-L-Rhap-(1→3)-α-L-Rha*p*-(1→2)-L-deoxy-Tal
20	*M. intracellulare*	2-*O*-Me-α-D-Rha*p*-(1→3)-2-*O*-Me-α-L-Fuc*p*-(1→3)-α-L-Rha*p*-(1→2)-L-deoxy-Tal
21	*M. avium*	4,6-*O*-(1-carboxyethylidene)-ß-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→2)-L-deoxy-Tal
25	*M. intracellulare*	2-*O*-Me-α-D-Fuc*p*NAc-(1→4)-ß-D-Glc*p*A-(1→4)-2-*O*-Me-α-L-Fuc*p*-(1→3)-α-L-Rha*p*-(1→2)-L-deoxy-Tal
26	*M. avium*	2,4-di-*O*-Me-α-L-Fuc*p*-(1→4)-ß-D-Glc*p*A-(1→4)-2-*O*-Me-α-L-Fuc*p*-(1→3)-α-L-Rha*p*-(1→2)-L-deoxy-Tal

Peptides and proteins are recognized by host immune system with a wide range of antigenic moieties using major histocompatibility complex (MHC) class I or II. The recognition of lipids is important for host defense against mycobacterial infection as well as other antigenic responses. Several bacterial lipid antigens were recognized by specific T cells. Mycolic acid derived from mycobacteria is lipid antigen that stimulates CD1b-restricted T cells [17, 18]. As other immunogenic mycobacterial glycolipids, glucose-6-monomycolate (GMM), PIM, and LAM are available for loading onto CD1 molecule and recognized by specific T cells [19, 20].

Cord Factor/TDM and Mycoloyl Glycolipids

Cord factor/TDM is prominent mycoloyl glycolipid and first isolated as toxic substance of virulent *M. tuberculosis*. After that, mycoloyl glycolipids have been found widely in acid-fast bacteria and exert potent biological and immunological activities. It has been clarified the unique structure of these mycoloyl glycolipids (Figure 3) and the pleiotropic activities, including the development of granulomatous inflammation, anti-tumor immune response, and adjuvant effects based on the induction of proinflammatory and type 1 helper T cell (Th1)-related cytokines from host cells [21-23].

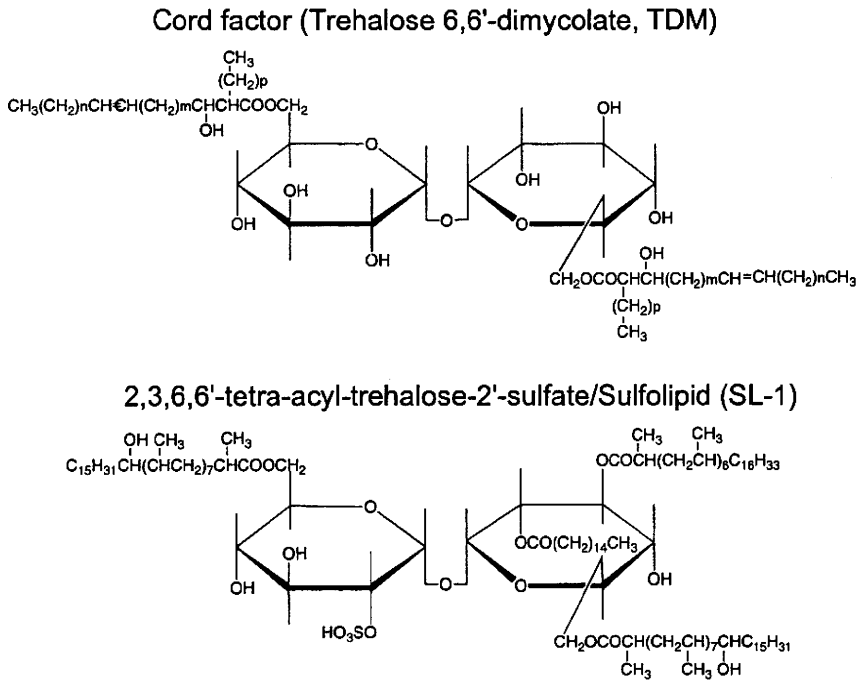

Figure 3. Proposed structures of cord factor/TDM and sulfolipid.

Cord factor/TDM is surface component of tubercle bacilli, and may contact with host in the early stage on infection. Administration of cord factor/TDM can induce delayed-type