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Recognition of *Treponema pallidum* and Other Spirochetes by the Innate Immune System

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1. Introduction

In 1905 the spirochete *T. pallidum* was discovered as the aetiologic agent of syphilis by Schaudinn and Hoffmann at the Charité Hospital in Berlin, Germany (Schaudinn & Hoffmann, 1905). This helical shaped bacterium is extremely well hidden, and also one of the best adapted to its only host – the Homo sapiens. The genome of *T. pallidum* ssp. *pallidum* contains only 1041 coding sequences and lacks numerous catabolic and biosynthetic pathways (Fraser et al., 1998) like, i.e. fatty acid synthesis (Livermore & Johnson, 1975). Therefore this organism utilises many of the biosynthetic precursors from its host and up to now it is not possible to continuously cultivate it *in vitro*. The only way to grow this bacterium is in *in vivo* models (Norris et al., 2006). Thus, the isolation of biological active compounds of *T. pallidum* has been difficult due to the lack of sufficient amounts of cultured bacteria. To study recognition of *T. pallidum* by the innate immune system information on the chemical composition of these cells has to be correlated with immunological responses induced by related spirochetes. The best examined spirochete is *Borrelia burgdorferi* – the etiologic agent of Lyme disease (LD). LD is an endemic disease with somewhat similar characteristics as compared to syphilis – A relatively slow dissemination of the spirochete within the host is followed by a weak inflammatory response of the human immune system. Furthermore, multiple organs are affected including the skin as well as the peripheral and central nervous system. Only half of the genes of *B. burgdorferi* code for proteins orthologous to those of *T. pallidum* indicating an adaptation to distinct niches though (Subramanian et al., 2000). However, the motility associated genes are highly conserved in both organisms (Fraser et al., 1998). Other pathogenic *Borrelia* include *B. hermsii*, one causative agent of relapsing fever, that multiplies more rapidly to higher cell numbers and causes more acute clinical symptoms. Further human associated treponemes such as *T. denticola*, colonise the oral cavity, *T. phagedenis*, belongs to the genital flora, and other species are found within the intestine. Thereof only the oral treponemes are pathogenic and have been associated with periodontal disease causing inflammation of the gingival tissue (Norris et al., 2006). Since the genome of *T. denticola* is much larger than that of *T. pallidum* and a conserved gene order could not be determined it is unlikely that *T. pallidum* is directly derived from this oral spirochete. But it might serve as a model for *T. pallidum* research since it is relatively easy to cultivate (Seshadri et al., 2004). While *Borrelia* and *Treponema* share the same phylogenetic family – *Spirochaetaceae* – the genus *Leptospira* belongs to the family *Leptospiraceae* in the same order as the first – *Spirochetales* (Paster et al., 1991). The most important and immunologically best studied leptospiral pathogen is the agent of Weil's disease (leptospirosis) *L. interrogans*.

1.1 The innate immune system

The innate immune system is the first line of defence of the host against invading microorganisms. Its function is to avoid an infection, or, in case an infection occurred, to detect, kill and eradicate the germs. Furthermore in vertebrates it interacts with the adaptive immune system and i.e. facilitates the presentation of antigens. The innate immune system mainly consists of either circulating or tissue resident cells, and humoral components like the complement system and cytokines. The phagocytes include the monocytes, macrophages, neutrophil granulocytes, or dendritic cells. These cells express germ-line encoded pattern recognition receptors (PRR) that detect conserved microbial structures not being present in the host. These receptor families include binding receptors like mannose binding receptors, CD14 or scavenger receptors like CD36. These proteins directly bind or mediate the binding of microbial patterns but they can't activate immune cells. The other PRRs are signalling receptors like toll-like (TLR), nod-like (NLR), or rig-I-like (RLR) receptors that usually contain a ligand-binding and a signalling domain. Upon ligand binding a conformational change within the signalling domain of the PRR triggers the signalling cascade inside the cell. This leads to the translocation of transcriptions factors into the nucleus and the release of cytokines (Akira & Takeda, 2004b).

1.2 The tools for receptor research

To assess the individual contribution of receptors of the innate immune response to a pathogen and the specificity of the ligands, loss-of-function and gain-of-function assays are used. There are mainly three ways to selectively disable single receptors in loss-of-function experiments. The most widely used system are knockout (KO) mice, in which receptor genes were turned off by homologous recombination in embryonic stem cells (Hemmi et al., 2000). Today numerous inbred KO mice are available either commercially or through research collaborations lacking relevant receptors or proteins of the signalling cascade (Akira & Takeda, 2004a). These animal models, however, do not always reflect the situation in humans. Genotyping of healthy volunteers for natural occurring functionally relevant mutations in the receptor genes allows experiments with isolated peripheral blood mononuclear cells from humans. Finally, loss-of-function experiments can be designed by downregulation of genes by small interfering RNA (siRNA) (Elbashir et al., 2001). Upon transfection of these plasmids into cells they interfere with the translation of the targeted mRNA leading to degradation of the mRNA prior to translation and a strong reduction of the receptor protein expression (knockdown). The most widely used assay for gain-of-function experiments are cell lines like the human embryonic kidney cells (HEK 293). In these epithelial cells numerous PRRs are either not expressed or expressed only dysfunctional while the signalling cascade is mostly intact. By transfection of receptor plasmids it is possible to study cellular activation upon stimulation with bacterial ligands in contrast to non-transfected cells. The read-out for these experiments are either cytokines like IL-8 or reporter-gene assays for transcription factors like NF-κB (Opitz et al., 2001). If working with novel isolated bacterial structures it is useful to first check their biological activity with cell lines that express a full set of PRRs. The most often used cell lines are human monocytes like THP-1 or the murine macrophages RAW 264.7 (Schröder et al., 2000).

1.3 The morphology and the cell wall composition of *T. pallidum*

Spirochetes stain negative in Gram-staining and share the main cell wall topology of Gram-negative bacteria. In detail the *T. pallidum* envelope is assembled by an outer and a cytoplasmic (inner) membrane enclosing the protoplasmic cylinder (Johnson et al., 1973).

The periplasmic space is constituted by a thin peptidoglycan layer (Umemoto et al., 1981) and anchors the endoflagella (also called axial filaments) (Johnson et al., 1973). From the centre of the cell the endoflagella wrap around the protoplasmic cylinder and extend at each end into the extracellular space (Hovind-Hougen, 1976). The outer membrane contains few transmembrane proteins (Jones et al., 1995; Radolf et al., 1989; Walker et al., 1989) and exhibits an extremely low protein/lipid ratio (Radolf, Robinson, Bourell, Akins, Porcella, Weigel, Jones & Norgard, 1995). Hydrophobic proteins are anchored in both membranes (Radolf et al., 1988). The majority, however, is located in the cytoplasmic membrane (Cox et al., 1995). Both membranes themselves are mainly constituted of lipids that comprise about 20 % of the dry weight of *T. pallidum* cells (Johnson et al., 1970). About 50 % of the total lipids are attributed to the glycolipid α-galactosyl-diacylglycerol (MGalD) (Livermore & Johnson, 1970) while about 45 % are phosphatidylcholine and -ethanolamine, which are found in the host too. The remaining portion are free fatty acids (Johnson et al., 1970). With more sensitive radiolabelling assays further phospholipids have been detected in minor proportions (Belisle et al., 1994). The peptidoglycan layer consists chemically of an oligomer of glucosamine and muramic acid that is cross linked by short peptides (Umemoto et al., 1981).

2. Toll-like receptors

Toll-like receptors (TLR) are single-pass transmembrane receptors. Within the cell one group is located on the cellular surface, the other within endosomes. They exhibit an ecto-domain containing leucine-rich repeats detecting the ligands, a transmembrane domain, and a cytoplasmic domain inducing signal transduction. This intracellular domain is termed toll/IL-1R (TIR) domain due to its homology to the IL-1 receptor signalling domain (Fig. 1, p. 4). Adaptor molecules associated with the TIR domain trigger intracellular signalling, with MyD88 being the central signal transducer (Akira & Takeda, 2004a). TLRs are not only expressed in cells of the innate immune system but partially in B lymphocytes and endothelial cells too. They are named according to their homology with the toll protein found in *Drosophila*. In 1997 the first human TLR was cloned and its function for the signalling of the immune system discovered (Medzhitov et al., 1997). Subsequently a protein family consisting of 10 members in humans was identified and numerous ligands proposed. Prior to these findings immunstimulatory molecules such as lipoproteins from *T. pallidum* (Norgard et al., 1995) or lipopolysaccharide from Gram-negative bacteria as well as the involvement of some binding receptors were established but the signalling receptor and the entire mechanism remained unknown.

2.1 TLR-4

TLR-4 is responsible for the recognition of lipopolysaccharides (LPS) (Poltorak et al., 1998; Qureshi et al., 1999) but binding assays revealed that MD-2, an accessory protein of TLR-4 receptor complex, directly binds LPS. Due to the association of MD-2 with a homodimer of TLR-4 it triggers signalling (Shimazu et al., 1999; Viriyakosol et al., 2000). Earlier it was found that the serum protein LPS binding protein (LBP) (Schumann et al., 1990) and membrane bound or soluble CD14 (Wright et al., 1990) are also involved in the recognition cascade of LPS. Both facilitate recognition of LPS by TLR-4 in the pg/ml range. All these membrane bound proteins are localised on the cell surface. LPS is an amphiphilic molecule that is located in the outer leaflet of the outer membrane of Gram-negative bacteria. Chemically it is composed of lipid A, a phosphorylated disaccharide with 4-6 attached fatty acids including hydroxy fatty acids, and the core region, an oligosaccharide with the characteristic carbohydrate

3-deoxy-D-manno-octulosonic acid (KDO). The active principle for binding to MD-2 and initiating signalling is lipid A (Viriyakosol et al., 2000).
Spirochetes share the cell wall design of Gram-negative bacteria but they seem to lack a classical TLR-4 stimulating LPS. This has been shown for *T. pallidum* (Hardy & Levin, 1983; Penn et al., 1985; Radolf & Norgard, 1988) as well as several *Borrelia* including *B. burgdorferi* (Hardy & Levin, 1983; Takayama et al., 1987). For *L. interrogans* an atypical LPS was reported (Vinh et al., 1986) and the chemical structure identified (Que-Gewirth et al., 2004). But this LPS is atypically recognised by TLR-2 instead of TLR-4 (Werts et al., 2001). In contrast several authors have reported on the putative isolation of LPS in *Treponema* (Kurimoto et al., 1990; Walker et al., 1999) and in *Borrelia* (Beck et al., 1985; Habicht et al., 1986). However, these findings are not convincing since the extracts are crude and not purified chemically. Furthermore, not all features of an LPS were found, and no chemical structure has been determined. Most importantly no activation of TLR-4 has been reported. Therefore it appears obvious that TLR-4 is not relevant in recognition of *T. pallidum* and spirochetes in general.

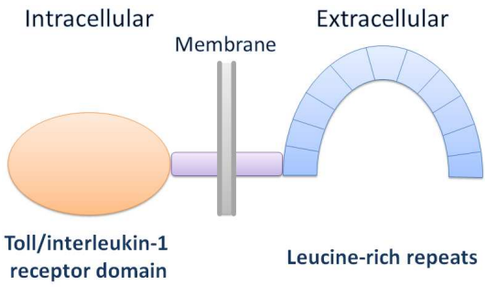


Fig. 1. Schematic representation of the basic structure of toll-like receptors

2.2 TLR-2, TLR-1, TLR-6

The first ligands described for TLR-2 were bacterial lipoproteins (Aliprantis et al., 1999; Brightbill et al., 1999; Lien et al., 1999). Later it has been shown that TLR-2 forms heterodimers with either TLR-1 or TLR-6 to recognise triacylated and diacylated lipoproteins, respectively (Takeuchi et al., 2001; 2002), and the crystal structures of both ligands in complex with the respective receptor dimer have been elucidated (Jin et al., 2007; Kang et al., 2009). For signalling via the TLR-1/-6 receptor complex CD36 is a crucial cofactor (Hoebe et al., 2005). TLR-1, -2 and -6 are located on the cellular surface as well (Kawai & Akira, 2009). Further ligands of diverse chemical nature have been proposed for TLR-2 and today it is the TLR with the highest number of proposed ligands. However, since the biological activity of bacterial lipoproteins and peptides in the upper pg/ml range (Schröder et al., 2004) is highest among the TLR-2 ligands, lipoproteins can be considered as the prototype TLR-2-ligand. The biological importance of bacterial lipoproteins is to anchor proteins into cellular membranes. Its chemical structure was first described in 1973 (Hantke & Braun, 1973). The basic structure of the lipid anchor is a diacylglycerol molecule that is thioether linked to a cysteine. The cysteine constitutes the N-terminal amino acid of the protein. In case of triacylated lipoproteins the N-terminal amino group is amide linked to a further fatty acid. The biosynthetic pathway is ubiquitous in bacteria and lipoproteins have been predicted in many bacteria (Madan Babu & Sankaran, 2002). The active principle for the recognition by TLR-2 heterodimers is the lipid anchor and not the protein moiety.

2.2.1 Bacterial lipoproteins in *T. pallidum*

Due to the complete genome sequencing and known signalling peptides numerous putative lipoproteins could be predicted in the microbes sequenced: 46 in *T. pallidum* (22 by Fraser et al. (1998)), 166 in *T. denticola* and 127 in *B. burgdorferi* according to new algorithms (Setubal et al., 2006). For *B. burgdorferi* it has been shown previously that their lipoproteins are triacylated (Beermann et al., 2000). Since in *T. pallidum* the necessary enzyme for the acylation of the N-terminus – apolipoprotein N-acyltransferase – is present[1], it is likely that its lipoproteins are triacylated as well. A fraction of lipoproteins from *T. pallidum* has been isolated (Chamberlain et al., 1989; Radolf et al., 1988) and shown to activate isolated murine macrophages and the cell line RAW 264.7 (Radolf et al., 1991). These results could be confirmed by the biological activity of fully synthetic lipopeptides (DeOgny et al., 1994) that are able to mimic inflammation *in vivo* (Norgard et al., 1995). This activity has been shown to employ a distinct receptor then LPS (Norgard et al., 1996) but involves CD14 (Sellati et al., 1998) and LBP (Schröder et al., 2004). Furthermore it has been demonstrated that the acylation of the lipid anchor is an essential feature for recognition (Morr et al., 2002; Radolf et al., 1995). The lipoproteins of *T. pallidum* and *B. burgdorferi* and their lipopeptide analogs have been used as a model to identify TLR-2 as the signalling receptor (Lien et al., 1999). However the heterodimer partner for *T. pallidum* lipoproteins remains undetermined. For oral treponemes the recognition of bacteria or cell wall components by TLR-2 has been reported (Asai et al., 2003). In case of *T. denticola* the heterodimer TLR-2/-6 is utilised for signalling indicating rather a diacylated lipoprotein (Ruby et al., 2007). Since lipoproteins of *T. denticola* have been described as activating murine macrophages prior to the knowledge of TLR-2 (Rosen et al., 1999) it is likely that they exhibit the same TLR-2 activity as *T. pallidum*. Probably lipoproteins are the most important TLR ligands of *T. pallidum* and spirochetes in general. In *B. hermsii* TLR-2 is crucial for the activation of the adaptive immune system and production of antibodies (Dickinson et al., 2010).

2.2.2 Glycostructures in treponemes

Polysaccharides have been isolated from treponemes and subjected to compositional analyses indicating many kinds of carbohydrates (Yanagihara et al., 1984). Amphiphilic glycostructures from the outer membrane of *T. denticola* were isolated but no defined chemical structure was elucidated (Schultz et al., 1998). Similar not further chemically purified glycostructures were obtained from culture supernatants of *T. maltophilum*, an oral treponeme, or extracted from the same cells. These have been shown to activate murine macrophages as well as cell lines in a TLR-2-dependent fashion but only in very high concentrations (μg/ml) (Opitz et al., 2001; Schröder et al., 2000). However contaminations with lipoproteins that have similar hydrophobic properties like these amphiphilic glycostructures could not be ruled out.

2.3 TLR-5

TLR-5 detects bacterial flagellin of several Gram-positive and Gram-negative bacteria. The receptor binds this protein directly and leads to NF-κB activation and release of proinflammatory cytokines (Hayashi et al., 2001; Smith et al., 2003). The recognised monomeric FlaA is highly conserved and a principle component of the flagellar filaments. It is essential for the motility of bacteria. Unlike other TLRs, TLR-5 is not expressed on macrophages or dendritic cells but mainly on intestinal cells (Uematsu et al., 2006). *T. pallidum* features several endoflagella that consist of flagellin too. The genetic analyses of the flagellar structure reveal that *T. pallidum* has three core proteins (FlaB1-3) and one sheath protein (FlaA) while in *B. burgdorferi* a single core and one sheath protein is found (Fraser et al., 1998). Of the

[1] http://www.ncbi.nlm.nih.gov/gene/6333053

spirochetes only for *B. burgdorferi* the role of TLR-5 has been assessed: Upon stimulation with live bacteria knockdown of the TLR-5 gene by siRNA resulted in either a minor effect (Dennis et al., 2009) or a significant reduction of cytokine gene expression (Shin et al., 2008). A direct stimulation of cells with the FlaA gene product (p37) increased the protein level of TLR-5 (Cabral et al., 2006). Taken the presence of FlaA in *T. pallidum* , its high conservation among bacteria, and the first results on the TLR-5 role in *B. burgdorferi*, TLR-5 probably contributes to the recognition of *T. pallidum* too. However, this fact and the extent of the signalling remains to be elucidated.

2.4 TLR-9

TLR-9 was identified as the PRR for unmethylated 2′-deoxyribo cytidine-phosphate-guanosine (CpG) DNA motifs (Hemmi et al., 2000). The general immune stimulatory effects of these motifs were already established prior to the discovery of TLR-9. In contrast to mammals in bacteria CpG DNA motifs are 20 times more common (Klinman et al., 1996). First it was assumed that the recognition is species specific via the nucleotide sequence of the CpG motifs (Bauer et al., 2001) while it was later revealed that the DNA carbohydrate backbone 2′ deoxyribose determines the activation of TLR-9 (Haas et al., 2008). This receptor is expressed in intracellular vesicles like endosomes. No results on the role of TLR-9 in the immune response to *T. pallidum* have been reported. For *B. burgdorferi* the production of proinflammatory cytokines in TLR-9 deficient murine macrophages was not diminished compared to wild-type macrophages (Shin et al., 2008). In contrast, the interferon α production induced by *B. burgdorferi* was significantly reduced upon TLR-9 inhibition (Petzke et al., 2009) and *B. hermsii* activates TLR-9 (Dickinson et al., 2010). However it remains an open issue whether also the DNA of *T. pallidum* could be recognised by TLR-9.

TLRs -3, -7, and -8 are located in intracellular compartments. They sense double (-3) and single stranded (-7, -8) RNA found in RNA viruses. Therefore these TLRs don't appear to be relevant for the innate immune recognition of spirochetes. For the human TLR-10 currently no clear ligand is known (Kawai & Akira, 2009).

3. Nod-like receptors

Nucleotide oligomerisation domain (NOD)-like receptors (NLRs) are a family of intracellular PRRs with 23 members in humans. They are expressed in many cell types but some primarily in phagocytes. The NLRs are multi-domain proteins consisting of a nucleotide-binding domain, leucine-rich repeats (LRR) and an N-terminal effector domain (Fig. 2). Similar to the TLRs the LRR bind the microbial structures while the effector domain triggers the signalling cascade leading to activation of mitogen-activated protein (MAP) kinase, translocation of NF-κB, or activation of the inflammasome (Franchi et al., 2009). The best characterised NLRs are NOD-1 and NOD-2 that sense substructures of the bacterial peptidoglycan (PG) (Ting et al., 2008).

3.1 NOD-2

NOD-2 has been revealed as the receptor for muramyldipeptide (MDP) from Gram-positive and Gram-negative bacteria. Upon binding of the ligand it triggers the activation of NF-κB pathway (Girardin et al., 2003b; Inohara et al., 2003). The immunstimulatory properties of MDP were known a long time before and it has been widely used as an adjuvant (in Freund's complete adjuvant) (Chedid, 1983). Furthermore it has been shown that MDP synergises with LPS in the induction of proinflammatory cytokine release (Takada et al., 2002; Wang

et al., 2001). NOD-2 is predominantly expressed in the cytosol of monocytes/macrophages (Girardin et al., 2003b). Chemically MDP is N-acetylmuramyl-L-alanyl-D-isoglutamine and it is the minimal glycosubstructure of PG also from spirochetes. For *T. pallidum* the basic MDP components muramic acid, alanine and glutamic acid[2] have been detected in its PG (Azuma et al., 1975). However the exact sequence of the glycan linking peptides and therefore the structural requirement for NOD-2 recognition in treponemes is still unknown. In one report the treponemal PG lacked the adjuvant activity to stimulate antibody production (Umemoto et al., 1981). Early reports on the biological activity of PG from *B. burgdorferi* (Beck et al., 1990) and from *T. denticola* (Grenier & Uitto, 1993) can't be included since the PG preparations were not devoid of lipoproteins and no specific receptors were assessed. Nevertheless for LD an important role of NOD-2 in an interplay with TLR-2 was demonstrated recently. Both receptors are necessary for an effective induction of cytokines by *B. burgdorferi* (Oosting et al., 2010). Also *B. hermsii* activates NOD-2 (Dickinson et al., 2010). Since *Borrelia* and *Treponema* are supposed to have the same PG composition the role of NOD-2 in the recognition of the syphilis spirochete should be examined.

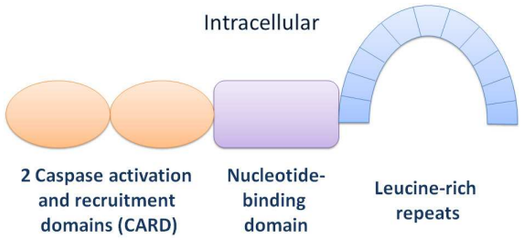


Fig. 2. Schematic representation of the structure of NOD-2 receptor

3.2 NOD-1

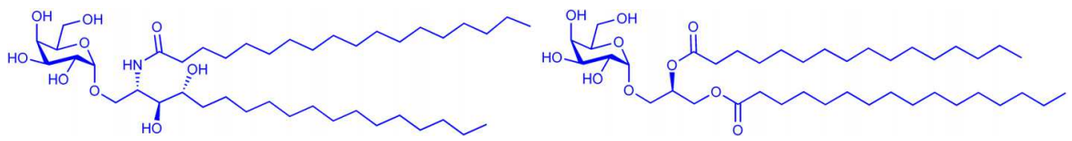
NOD-1 is the sensing PRR for another PG motif, the iE-DAP dipeptide of the glycan strand cross linking peptide (Chamaillard et al., 2003; Girardin et al., 2003a). The receptor-ligand complex then leads to the activation of NF-κB and cytokine release as well. NOD-1 is expressed in the cytosol of multiple tissues (Chamaillard et al., 2003). The unique part of the ligand iE-DAP (γ-D-glutamyl-*meso*-diaminopimelic acid) is the diaminopimelic acid. In general all common Gram-negative and only several Gram-positive bacteria exhibit this diamino acid. In spirochetes it was only detected in *Leptospira* while in contrast *Treponema* and *Borrelia* contain the diamino acid ornithine instead (Umemoto et al., 1981; Yanagihara et al., 1984). Furthermore, for *B. burgdorferi* it has been shown that NOD-1 plays no major role in the recognition by the immune system (Oosting et al., 2010) and the same is expected for *T. pallidum*.

4. CD1d

CD1d is a surface glycoprotein similar to the MHC class I molecules that presents lipid antigens to invariant natural killer T cells (iNKT). The iNKT cells are a small subset of T lymphocytes that express an invariant $\alpha\beta$ T cell receptor as well as a NK cell receptor. They activate monocytes and B-cells by immunregulatory cytokines linking the innate and adaptive immune system (Cohen et al., 2009). This seems to be an important mechanism for production of antilipid antibodies too (Leadbetter et al., 2008). For LD it has been shown

[2] during the applied compositional analysis isoglutamine will be converted to glutamic acid

that CD1d deficiency impairs the host resistance to *B. burgdorferi* in a mouse model (Kumar et al., 2000). In line with this is the observation that iNKT deficient mice exhibit more severe and prolonged manifestations as well as a reduced ability to clear the spirochetes (Tupin et al., 2008). This effect could be attributed to one of the *B. burgdorferi* glycolipids namely α-galactosyl-diacylglycerol (MGalD) (Fig. 3b) that activates iNKT cells via CD1d and independent of TLRs (Kinjo et al., 2006). MGalD is structurally related to the prototype iNKT cell ligand α-galactosylceramide (Fig. 3a) and shows the essential α-configuration of the galactose (Kawano et al., 1997). Furthermore for tick-borne relapsing fever it was demonstrated that CD1d deficiency coincides with impaired antibody production and increased *B. hermsii* burden (Belperron et al., 2005). Due to the fact that *B. hermsii* lipids contain MGalD too (Livermore et al., 1978) a crucial role of CD1d and its ligand MGalD in the defence of *Borrelia* can be concluded. For syphilis the role of CD1d and iNKT cells have not been assessed so far. However while in *B. hermsii* and *B. burgdorferi* MGalD comprises for 2.6 % and 3.4 % of cell dry weight respectively (Stübs et al., 2009) in *T. pallidum* MGalD is the major lipid structure accounting for 9-10 % of the dry bacterial cell (Johnson et al., 1970; Livermore & Johnson, 1970). Therefore we hypothesize that for syphilis the activation of iNKT cells by CD1d and treponemal MGalD is an important mechanism for the innate as well as the adaptive immune response.



(a) α-Galactosylceramide (b) α-Galactosyl-diacylglycerol

Fig. 3. Chemical structures of CD1d ligands

5. Phagocytosis

In vivo the host's immune cells are faced first with live bacteria which do not expose the epitops for the PRRs to the surface. For *B. hermsii* the crucial role of the initial bacterial cell degradation has been demonstrated: Blocking of internalisation of the bacterial cell prevents the induction of inflammatory cytokines (Dickinson et al., 2010). Hence, prior to TLR/NLR recognition and signalling an effective phagocytosis of bacteria is important. At first the phagocytes have to rely on their low affinity binding receptors and the complement opsonins to attach the bacteria. In a later stage of the immune response highly specific IgG antibodies can opsonise the bacteria and allow the phagocytes to bind their F_C moiety with much higher affinity. Thereafter immune cells ingest and lyse the bacteria in the phagolysosome. For live *T. pallidum* the phagocytosis proceeds considerably slower than with other bacteria (Alder et al., 1990). Compared to *B. burgdorferi* it results in significantly weaker activation of monocytes and less release of cytokines. Only in the presence of syphilic sera *T. pallidum* initiates an equally efficient immune response as compared to *B. burgdorferi* (Moore et al., 2007). For the uptake of *B. burgdorferi* the adaptor molecule MyD88 but not TLR-2, -5 and -9 is important and plays a dual role – for signal transduction and for phagocytosis (Shin et al., 2008). Breaking down the bacterial polymers to small subunits is not only obvious for the mentioned NLRs but also for TLR-5. Here the acidic environment in the phagolysosome is necessary to disperse the filament into the monomeric flagellin (Smith et al., 2003).

6. Conclusion

The first crucial step for immune responses upon infection with *T. pallidum* is its degradation in order to have the ligands available for PRRs of the innate immune system. However, during early syphilis IgG antibodies are absent and phagocytosis of *T. pallidum* is markedly weak. Therefore only limited amounts of treponemal ligands are recognised by PRRs rendering low inflammation. Furthermore pyrogenicity of *T. pallidum*, lacking the highly active LPS, is diminished in regard to Gram-negative bacteria. The most potent PRR ligands in *T. pallidum* are lipoproteins recognised by TLR-2. Compared to *B. burgdorferi* the number of distinct lipoproteins is more limited but no conclusion as to the overall expression can be drawn. Since the activation of the innate immune system by *T. pallidum* and *B. burgdorferi* is similar in extent, it appears that TLR-5, recognizing flagellin, and TLR-9, recognizing DNA, play a role for recognition of *T. pallidum* as well. Both ligands are present in *T. pallidum* from the genetic and chemical point of view but remaining uncertainty has to be assessed by experiments with treponemal ligands. The same holds true for the activation of immune cells by MDP via NOD-2. Chemical analyses indicate the presence of the MDP motif but further evidence has to be gathered. The role of TLR-1 and -6 as heterodimers of TLR-2 have not been elucidated finally. However, the presence of triacylated lipoproteins in *T. pallidum* rather suggests a function of TLR-1. The other TLRs and NOD-1 are probably not involved in *T. pallidum* recognition. The presentation of treponemal MGalD by CD1d to iNKT cells is a novel aspect presented here first and should be studied in detail.

Thus, the weak phagocytosis combined with the reduced recognition of *T. pallidum* by the PRRs can explain its "stealth" during the first stages of syphilis. In the absence of IgG antibodies *T. pallidum* induces only weak inflammation and leads to painless ulcerations as in the primary stage. Insufficient recognition and eradication enables *T. pallidum* to disseminate by the blood stream and lymphatic system and affect other organs as in the second stage of syphilis. Furthermore the weak activation of the innate immune system results in diminished presentation of antigens for adaptive immune responses. The following delayed production of IgM and more pronounced IgG antibodies has been observed by serodiagnostics. However, in late stage syphilis IgG antibodies are present and an efficient phagocytosis has been demonstrated *in vitro*. At this time *T. pallidum* can only persist in the host due to evasion into organs with restricted immune responses as the central nervous system. In late syphilis dissemination of *T. pallidum* within the host is prevented and the host is not infectious anymore.

Taken together different characteristics of *T. pallidum* allow it to evade the immune response of the host – passively to disseminate during early syphilis and actively to establish a chronic infection. For both features the interaction with the innate immune system is pivotal.

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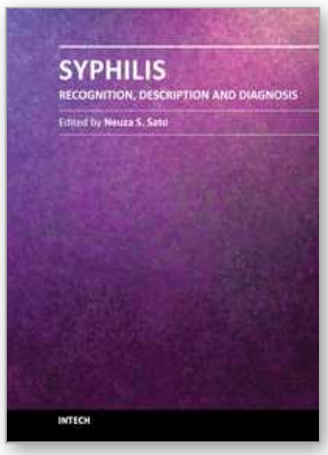

Syphilis - Recognition, Description and Diagnosis
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Syphilis, a sexually transmitted disease was first described in 15th century, is caused by Treponema pallidum subsp. pallidum and occurs worldwide. This book is a collection of chapters presenting the novel knowledge about the T. pallidum and some historical and up to date information about venereal disease and syphilis. The collection of articles includes: immunological aspects recognition of T. pallidum by the pattern recognition receptors of the innate immune; the whole genome analysis of treponemes and new targets for its molecular diagnosis; some historical aspects of venereal diseases treatment; natural history of syphilis including clinical manifestation and epidemiology; a clinical aspects dealing with psychiatric manifestations of neurosyphilis; spatial and temporal patterns of primary syphilis and secondary syphilis described by the spatial and space-time scan statistics; a commonly used methods for laboratorial diagnosis, the serological response to treatment of syphilis and safety in blood transfusion. I hope this book will be useful for students and research fellows as well for the wide audience.

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