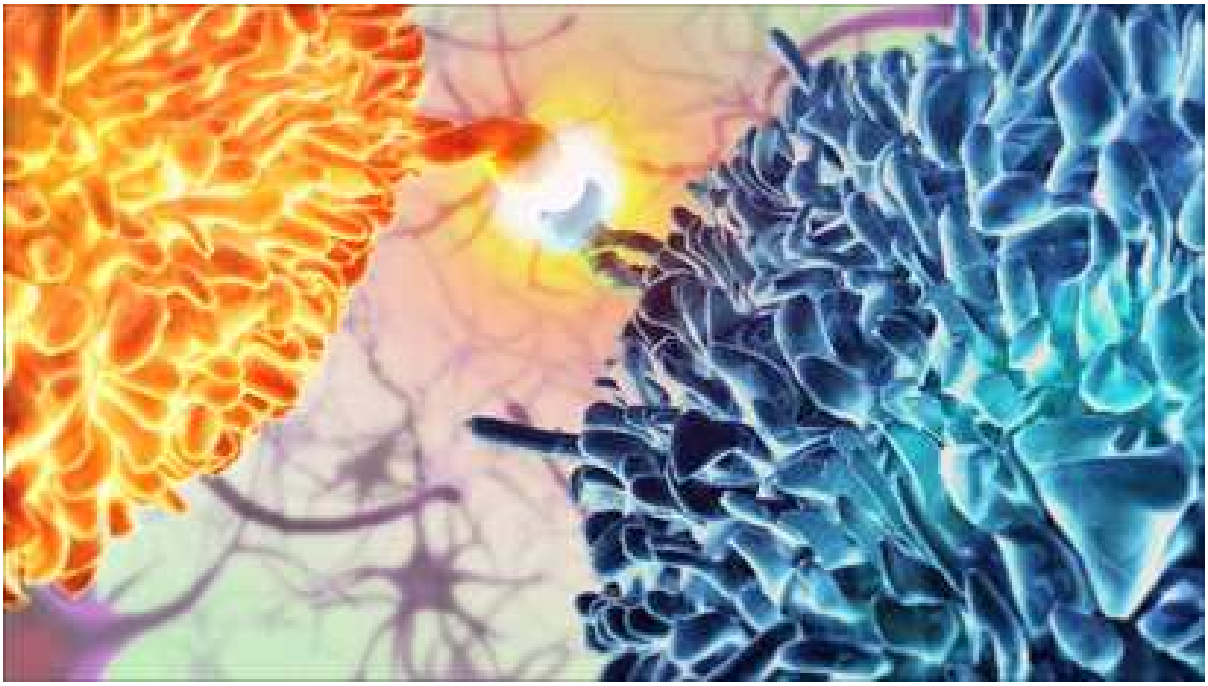
Antigen presentation. How sweet?!!



Thesis to complete the master programme “Immunity and Infection”

Author: Ewaldus Bernardus Compeer, BSc
Supervisor: Marianne Boes, PhD, Associate Professor
Reviewer: Debby Bogaert, MD, PhD

Date: December 2009 - January 2010

Abstract

Thus far, the best-studied and most appreciated biopolymers are the proteins. Although the investigation of proteins has been shown to be necessary for understanding of biological phenomena, more and more evidence shows that biological events cannot be explained solely by the functions of proteins. Fine tuning of the system requires the involvement of other biomolecules, such as carbohydrates and lipids. Key to our specific defense against microbial infections are critical interactions between the specific adaptive and relatively non-specific innate part of the immune response. The most critical link between both parts of the immune system is T cell activation by antigen presentation. This report describes the immunological significant role of bacterial-derived carbohydrate structures in T cell activation, as context of antigens as well as antigen itself. This report will expand the concepts of the role of carbohydrates in microbial interactions with the adaptive immune system.

Content

Abstract 1
Content 2
Introduction 3
§1: Classical antigen presentation via Major Histocompatibility Compex class II molecules 3
§2: Antigen presentation via MHC class I molecules 6
§3: Canonical antigen presentation via MHC class I molecules: cross-presentation 10
Contribution to antigen presentation of bacterial-derived carbohydrates as the context of antigens. 14
§1: Carbohydrate structures present on bacteria 14
§2: Recognition of bacteria via their carbohydrate structures. 15
§3: Consequences of extracellular recognition of bacterial-derived carbohydrates on bacterial uptake. 19
Conclusion: 21
§4: Contribution of carbohydrate structures present on bacteria to intracellular antigen sorting. 22
§5: Contribution of bacterial-derived carbohydrate structures recognition to T cell activation 25
Zwitterionic capsular polysaccharides: T-cell dependent antigens 27
§1: Immune response to capsular polysaccharides 27
§2: Zwitterionic polysaccharides 28
§3: ZPS structure 29
§4: ZPS processing 31
§5: Immunomodulatory role of ZPSs in intra-abdominal abscesses 34
§6: Contribution of zwitterionic polysaccharides to antigen sorting 36
Discussion 38
References 42

Introduction

The induction of immune responses requires critical interaction between innate parts of the immune system, which respond rapidly and in a relatively nonspecific manner, and other specific parts, which recognize particular epitopes on an antigen. The critical link between the innate part and the specific part of the immune response is presentation of encountered antigens to the adaptive part to induce an immune response. The consequential immune response must be restricted to dangerous antigens and optimized to the presented antigen to prevent over-reactive or self-reactive immune responses. The presentation platforms for antigens are the Major Histocompatibility Complex molecules, which are able to present both exogenous and endogenous encountered antigens. Cells are constitutively internalizing compounds from their environment by different processes, including phagocytosis, endocytosis, and macropinocytosis, thereby encountering a lot of different compounds. However, only dangerous compounds must be selected to induce a specific immune response by the adaptive immunity. Since the main constituent of the bacterial surface are carbohydrates, it is not unexpected that these biomolecules can be recognized by the immune system as harmful and dangerous molecules. This report will discuss the contribution of the bacterial-derived carbohydrates in the development of a specific and efficient adaptive immune response and will especially focus on the current knowledge on antigen presentation and the role of bacterial-derived carbohydrate structures in this process.

§1: Classical antigen presentation via Major Histocompatibility Compex class II molecules

MHC II expression.

As mentioned previously, the Major Histocompatibility Complex (MHC) class II is essential for a proper immune response against invading pathogens. The expression of MHC class II molecules is under steady state conditions restricted to a specific group of cells, named antigen presenting cells (APCs). Cells that are more specialized to acquire, process, and present antigens are the so-called professional antigen presenting cells. This group comprises cells from the monocyte-macrophage lineage, dendritic cells (DCs), and B lymphocytes. The DCs constitutively express MHC class II molecules and co-stimulatory molecules that are essential for T cell activation and proliferation as a function of their developmental stage [1]. In contrast, expression of MHC class II and co-stimulatory molecules is potently induced upon activation in macrophages, while B cells constitutively express MHC class II molecules but require stimulation to express co-stimulatory molecules. The expression of MHC II requires the transcriptional co-activator called the MHC II transactivator (CIITA) [2]. Next to the professional antigen presenting cells, also other cell types can express MHC class II molecules upon stimulation. These cells are called non-professional antigen presenting cells, to emphasize that these cells are not specialized to present and process acquired antigens. Non-professional antigen presenting cells include, fibroblasts, glial cells, thymic epithelial cells, and pancreatic B cells [3].

Human MHC class II molecules are encoded by polymorphic genes clustered on the short arm of chromosome 6, named “human leukocyte antigen-DR, -DP, -DQ, and two non-classical MHC II genes, HLA-DM and -DO[4,5]. In mice two major groups of MHC class II genes exist, I-A and I-E, as well as non-classical H-2M and H-2O.

To date, more than 870 MHC II allotypes have been identified in the human population, from which approximately 600 proteins were expressed. This high polymorphism produces a great diversity of peptide receptors and enables the species to survey an amazing variety of peptide sequences derived from the multitude of pathogenic organisms [6].

MHC II assembly.

MHC class II complexes are composed of two non-covalently linked type I membrane polypeptides, subunit α (35kDa) and β (28kDa) respectively. After synthesis and translocation into the endoplasmic reticulum (ER), both subunits are forced into correct assembled heterodimeric complexes by their transmembrane domain [7] and associating invariant chain that function as a chaperone[8]. The invariant chain and MHC II subunits associate into a nonameric complex, of which

the precise order of assembly is not clear. Probably the invariant chain self-associates into trimers prior to association with either preassembled αβ MHC class II complex or single α and β subunits. By binding to the MHC II complex, the invariant chain prevents binding to MHC II of endogenous peptides that are present in the ER [9]. Another function of the invariant chain is to match isotypic α and β-chains. This is necessary since the α- and β-subunits are not examined by antigenic peptides in the ER to form functional peptide receptors, because MHC class II heterodimers do not acquire antigenic peptides in the ER as MHC class I molecules does [6].

After proper assembly in the ER, the nonameric complexes pass through the Golgi cisternae into the TGN from where the complexes are delivered in the endocytic compartment as dictated by the bound invariant chain[10]. However, the precise route of the MHC II-invariant chain complexes from TGN to the endocytic compartment that is called MHC class II-containing compartments (MIIC) remains an unsolved question. In the literature, some reports argue the prevalence of a direct, intracellular transport from the TGN to either early or late endosomes and then to lysosomes[11], whereas other studies suggest an indirect pathway from the TGN to the plasma membrane, followed by endocytic delivery to early endosomes, late endosomes, and finally lysosomes[12].

MHC II-peptide complex formation.

Upon encounter of an invading pathogen by an APC, innate receptors recognize and bind to the pathogen. Binding of pathogens initiates waves of signal transduction events that induce rearrangement of the actin cytoskelet leading to internalization of the pathogen into phagosomes. The phagosome develops or matures into a phagolysosome by fusing with endosomes or lysosomes, thereby intersect with the MHC II complex routing. The exposure of antigenic proteins and MHC class II molecules to endosomal and lysosomal-residing proteases is required for peptide loading into MHC class II molecules.

Whereas the α and β chains are resistant to proteolysis, the invariant chain undergoes proteolytic degradation by cysteine and aspartic proteases, including cathepsin S, to various fragments in the endocytic compartment [13-15]. One of these fragments, the approximately 3-kDa class II-associated invariant chain-derived peptide (CLIP), remains bound to the peptide-binding groove on the surface of the αβ dimers[16]. CLIP is eventually exchanged by antigenic peptides in a process that is catalyzed by HLA-DM and HLA-DO.

The MHC class II-like molecule HLA-DM forms a stable heterodimer of DM α-chain and DM β-chain proteins, encoded by HLA-DM and HLA-DMB genes. Unlike classical MHC class II molecules, HLA-DM is not expressed at the cell surface but resides intracellular in the MIICs [17]. Crystallography studies of HLA-DM or the mouse variant H-2M show an overall structure that is similar to classical MHC class II molecule structures, but lacks a functional peptide-binding groove[18,19]. Further investigation demonstrated that HLA-DM first releases CLIP from the class II groove[20], then stabilizes the empty class II molecules[21], and finally shapes the repertoire of peptides that are loaded by biasing the peptide pool that binds MHC class II molecules[22]. The peptide loading activity of HLA-DM has an acidic pH optimum and involves physical interactions, thus the association and related function of HLA-DM to MHC II complex is localized to a later stage in maturation of endocytotic vesicles in which the pH is acidified.

The HLA-DO and H-2O gene products are structurally similar to conventional class II molecules, even more to classical MHC class II molecules than to the HLA-DM proteins[23]. In the ER both α and β subunits of HLA-DO weakly dimerize and only after HLA-DM(αβ) binding this complex can exit the ER to localize in the MIICs mediated by a tyrosine-based motif (YTPL) in both β chains[24]. This association is maintained during and after transport to the MIICs [25]. Unlike classical MHC class II molecules and DM/H-2M, which are constitutively expressed by B cells, DCs, and macrophages, HLA-DO and H-2O have a more restricted expression pattern. They are being expressed on B cells, thymic epithelial cells and some subsets of DCs, but not on macrophages [26,27]. I*n vitro* it was proved that HLA-DMDO complexes were less efficient in peptide loading onto HLA-DR (classical MHC II), indicating that HLA-DO could inhibit HLA-DM functions [28]. Moreover, HLA-DO inhibition on HLA-DM function was more prominent at pH 5.5,

whereas the HLA-DODM complexes regain ability to catalyze peptide loading at pH less than 5.5[25,27]. This indicates that HLA-DO preferentially inhibits HLA-DM activity in early endocytic compartments (pH greater than 5.5), while allowing efficient peptide loading in late endosomal and lysosomal MIIC compartments (pH less than 5.5).

Other factors that determine peptide loading are the interactions between antigenic peptides and MHC class II molecules. The peptide-binding sites of MHC II molecules are comprised of an eight-stranded β- sheet topped by two long α-helical segments, forming an elongated binding groove for a diverse repertoire of peptides or polypeptides. The size of the groove favors peptides of 12 to 20 amino acids in length, which protrude from both ends of the groove, and are held in the groove by two different types of intermolecular interactions. The first is a co-operative network of hydrogen bonds that is formed between the peptide backbone and side chains of the α-helical regions flanking the peptide, tethering the peptide at multiple sites in a sequence-independent fashion. The second type of interaction involves peptide side chains fitting into specificity pockets lining the groove, which imparts sequence specificity. Peptide-dissociation experiments with mutant MHC II molecules and with side chain- or backbone-modified peptides indicate that both side chain and hydrogen bond interactions are critical for the stability of the peptide/MHC II complex [29]. The peptide-binding groove protects the core peptide from further proteolysis.

After peptide loading, the MHC II-peptide complexes are localized via tubular extensions that emanate from late endosomal compartment to the plasmamembrane. During endosomal maturation a gradual accumulation of luminal vesicles (LV) that bud off inward from the endosomal delimiting membrane occurs, leading to the formation of so-called multivesicular bodies (MVBs). One step further in endosome maturation, these MVBs fuse with lysosomes delivering their cargo to the destructive environment of lysosomes. The degradation of the luminal vesicles contribute to the formation of multilaminar membranes that are characteristic for the end-product of lysosome-MVB fusion. The MHC II-peptide complex carrying tubular extensions radiate out from both MVBs and multilaminar lysosomes[30,31]. It is unclear whether the tubules itself or vesicles that derive thereof fuse with the plasmamembrane, but the absence of secreted soluble lysosomal enzymes and the demonstration of vesicles at the tip of the tubular extensions favors the vesicle model[30]. By using spinning disk confocal microscopy, it was demonstrated that the tubular extensions have bidirectional movements, that requires microtubules and microtubule-associated motor proteins, indicating an active transport towards the plasmamembrane[32]. Indeed, T-cell engagement by peptide presentation of DC was shown to rapidly rearrange the endolysosomal tubules towards the point of contact between both cells, e.g. future immunological synapse [33]. The retention of of LAMP-1 in the lysosome, indicates a selective entering into the tubular extensions of MHC class II molecules [32].

Eventually the MHC II-peptide complex is presented on the cellular surface, where the peptide can be recognized by T cell receptor (TCR) of helper T cells ($CD4^+$), driving antigen-specific T cell activation. In contrast to B cell receptors (BCRs) of B cells which are capable of recognizing an antigen alone, T cell receptors only recognize antigen that has been processed and presented in the context of the MHC class II molecule.

T cell activation occurs in secondary lymphoid organs, such as lymph nodes, whilst antigen encounter occurs at the barriers of the body. This indicates that the antigen have to be delivered to the T cells. Indeed, DCs are known to migrate upon encounter of the antigen towards specific compartments in lymph nodes to activate T cells. Upon encounter of an antigen immature DCs undergo dramatic changes which in general result in decrease of uptake-capability, but more specialization into capabilities for antigen presentation. This process is called DC maturation. The DCs also provide additional information to program the lymph node-residing T cells to specialized susbets to optimize the immune response against the encountered antigen. Examples of specialized helper T cell subsets are Th1 cells that are balancing the immune response towards the cellular immunity, whereas Th2 cells balance the immune response towards the humoral immune immunity.

MHC II recycling.

In DCs, MHC II is constitutively synthesized and loaded with peptides from endogenous or exogenous acquired antigens. The amount of peptide-loaded MHC class II molecules at the cell surface needs to be tightly regulated. To regulate the amount of MHC II-peptide complexes present on the plasmamembrane MHC II-peptide complexes can be internalized, e.g. removal from the plasmamembrane. Efficient endocytosis of MHC II-invariant chain complexes is known to largely rely on the dileucine-based signals in the cytoplasmic tail of the invariant chain. But for the generation of MHC II-peptide complexes is the invariant chain (or CLIP) exchanged for the peptide and thereby lost the dileucine-based signals. However, studies showed that MHC II-peptide complexes that lack these signals are nevertheless endocytosed by immature DCs [34]. The removal of this dileucine-based signal from the MHC II β-chain did not completely abolish MHC II-peptide complex internalization. Studies have found a second endocytosis signal for MHC II-peptide complexes, which is recently identified as an oligo-ubiquitin tag. The oligo-ubiquitin tag are 3 to 5 ubiquitin moieties that are attached to a unique and between species and haplotypes highly conserved lysine residue (K225)[35]. Likely ubiquitin-interacting motifs of ubiquitin-binding clathrin adaptors target membrane proteins for endocytosis with clathrin-coated vesicles.

At endosomes, ubiquitinated membrane proteins are recruited by a sorting machinery, the Endosomal Sorting Complex required for Transport (ESCRT) for sorting to luminal vesicles in MVBs. Directing it to degradation by lysosomal content after fusion of MVBs with lysosomes. The precise regulation is of MHC II ubiquitination is not known. For attachment of ubiquitin on proteins, devoted so-called E3 ligases are essential. Recently, it was demonstrated that overexpression of an E3 ligase that is expressed in human DCs, i.e. MARCH1, promotes targeting of MHC II to lysosomes[36]. However, ubiquitination of MHC class II molecules in DCs from an MARCH1-knock out mice was not completely ablated, suggesting also other factors involved [37]. Moreover, only MHC II-peptide complexes are ubiquitinated and prior degradation of the associated invariant chain is required [35], suggesting that E3 ligases can only interact with MHC class II molecules after the release of CLIP.

The amount of cell surface presented MHC II-peptide complexes depends on the DC maturation stage. The low amount of MHC II-peptide complexes characteristic for immature DCs is regulated in part to their ubiquitination that targets them for degradation in lysosomes. During maturation of the DC upon activation by pathogens or inflammatory stimuli, the MHC II-peptide complexes are less efficiently ubiquitinated, leading to an increased presentation of peptide-loaded MHC class II molecules on the DC. Supported by the fact that MARCH1 is downregulated during DC activation [38].

MHC class II molecules need to encounter peptides prior to assembling the MHC II-peptide complex. A cell is constitutively internalizing molecules and even fluids with processes as macropinocytosis, receptor-mediated endocytosis, and phagocytosis. This give rise to a large amount of vesicles that are inside the cell, indicating that efficient delivery of antigenic peptides derived from internalized pathogens to MHC class II containing compartments require some sort of targeting.

§2: Antigen presentation via MHC class I molecules

MHC I expression..

MHC class I molecules display a 'peptide fingerprint' of intracellular protein content to cytotoxic T cells (CD8+) for immune surveillance of viruses and tumors. The MHC class I–peptide complexes are monitored by cells of both the innate and acquired immune systems, namely natural killer (NK) cells and $CD8^+$ cytotoxic T lymphocytes (CTL), respectively. In contrast to the MHC class II molecule expression that was restricted to APCs, MHC class I molecules are expressed on most nucleated cells, although the level of expression differs among different cell types. The class I molecule contain a 45 kDa α chain associated with a 12 kDa β_2-microglobulin molecule. In humans, classical MHC class I α chains are encoded from HLA-A, -B, and –C. In mice the classical MHC I α chain is encoded from genes designated H-2K,-2D, and -2L. Note that H-2L is only expressed in some mouse haplotypes. The β_2-microglobulin molecule is encoded on another chromosome from a highly conserved gene fragment.

Ubiquitin-dependent proteasomal degradation.
In steady state, cells degrade misfolded or defective ribosomal products that are present in the cytosol of cells by the ubiquitin-proteasome pathway. During infection, pathogen-derived proteins are expressed at high levels, increasing their proteasomal degradation compared to the host protein turnover[39].
Since both endogenous self antigens as well as non-self antigens (such as viral proteins) are residing in the cytosol, the discrimination between 'self' and 'non-self' proteins is crucial to avoid autoimmunity. Specific targeting of proteins, both self and non-self, to proteasomal degradation is mediated by the ubiquitin system. Ubiquitin is a highly evolutionarily conserved polypeptide of 76 amino acids. For conjugation of ubiquitin to protein substrates at first ubiquitin activating enzyme E1 generates a high-energy thiol ester intermediate in an ATP-dependent reaction. Subsequently, E2 ubiquitin-carrier or ubiquitin-conjugating proteins (UBCs) catalyze the transfer of the activated ubiquitin from E1, via an additional thiol ester intermediate (E2-S-ubiquitin), to the substrate that is specifically bound to a member of the ubiquitin-protein ligase E3 family. This ubiquitin is predominantly conjugated to the NH2-terminal amino group of the substrate. Repeatedly adding activated ubiquitin moieties to internal lysine residues (Lys-21, or -22) on the previously conjugated ubiquitin molecule generates a poly-ubiquitin chain that targets the substrate protein to the proteasome. The specificity of this targeting is predominantly coordinated by the E3 family. These recognition factors decide which specific substrate protein will be poly-ubiquitinated, allowing recognition by the 19S part of the proteasome. However, in most situations the substrates are not immediately recognized by E3s. In most cases the E3 requires posttranslational modifications or the substrate undergoes certain changes that render it susceptible for recognition.
In the literature many reports describe foreign peptides processed by the 26S proteasome as it is the main protease for creating MHC class I ligands. The main eukaryotic proteasome is essential in non-lysosomal ubiquitin-dependent protein degradation and is composed of two 19S ATPase regulatory particle, which is responsible for recognition, unfolding and translocation of ubiquitinated proteins, and the 20S core particle containing the proteolytically active centres[40]. The 20S core particle is a complex containing 28 subunits; four rings containing seven members that are stacked on top of each other[41]. Fourteen β subunits form the inner cavity and contain the proteolytically active sites, whilst 7 α subunits are situated at each end of the rod-like structure. The proteasome is a non-specific protease that can cleave after all amino acid residues [42], thus to avoid degradation of proper folded and functioning cellular proteins the activity of the proteasome needs to be tightly regulated. The specificity is regulated by the α subunits. Access to the inner cavity where the active sites are situated is only possible through the outer ring formed by the α subunits. Opening and closing of the central pore in the α-ring is regulated by proteasomal activators. One of the proteasomal activators is the well-known 19S ATPase particle. The 19S complex is necessary for the processing of both ubiquitin-dependent and -independent degradation. The complex comprises at least 18 different subunits that are topologically arranged the "base"and the "lid". In addition to controlling the gating of the 20S proteasome, the ATPase ring function as a chaperone for final unfolding of substrates and their translocation into the 20S proteasome catalytic inner cavity. Other accessory molecules are the 11S activating complex, the proteasome activator 28, and the inhibitory proteins proteasome inhibitor 31 (PI31) and Pr39, respectively. The proteasome inhibitors function through binding to the α7 subunit, thereby reducing proteolysis of specific proteins without altering global cell proteasomal proteolysis [43].
After stimulation by proteasomal activators, the opening is just wide enough to allow access of selected and unfolded polypeptide chains. The proteasome produces a large number of peptides in the course of ATP-dependent protein degradation with an average length distribution of 2–25 amino acids. Recently, it was discovered that the production of antigenic peptides can also be achieved by splicing of two distinct peptide fragments [44]. For peptides to be cut and re-ligated, the precursors must be compartmentalized within the catalytic unit and highly concentrated at the active sites to avoid interference from other competing peptides and active water molecules, making it a rare event [45]. Thus this peptide splicing increases the variability of the peptide pool.

Another way to increase the variability of the peptide pool for the proteasomal degradation process is substitution of the constitutive active subunits of the proteasome by their immunological counterparts. It was found that alternative β forms denoted β1i, β2i, and β5i can be expressed in hematopoietic cells in response to exposure to pro-inflammatory signals such as cytokines as TNFα and IFNβ, but in particular IFNγ. This proteasome composed of these alternative subunits is known as the immunoproteasome. Analysis of peptide fragments generated in vitro showed that the presence of immuno-subunits altered the cleavage site preference of proteasomes. This resulted in a more frequent usage of specific cleavage sites with the consequence that the relative abundance of certain peptides within the generated peptide pool is changed.

In addition, stimulation of cells with IFNγ not only induces the expression of immuno-subunits but also that of PA28αβ. PA28 is even in absence of cytokine stimulation found in almost any cell type, but increased constitutive levels are observed after stimulation with IFNγ in cells with specialized antigen presenting function[46]. PA28 attaches in an ATP-independent way to the outer α-rings of the 20S proteasome and strongly stimulates the hydrolysis of short fluorogenic peptide substrates. However, the turnover rate of cellular native proteins is not affected[47]. Crystallization studies revealed that PA28 induces conformational changes via its C-termini in the N-terminal parts of the α-subunits, thereby opening the central gates in the two outer α-rings of the 20S core complex. Based on this and on the observation that PA28 facilitates product exit, it was proposed that such an open conformation might allow the release of slightly longer peptides and, in consequence, might support the presentation of MHC class I antigen presentation[48].

These findings reveal that cells possess efficient mechanisms for improvement of control over the quantity and quality of antigenic peptides presented by MHC class I molecules. Since most peptides generated have high affinity for MHC class I molecules, there are almost unlimited possibilities for antigen presentation on the cell surface and essential for the adaptive immune responses. The peptide products of the proteasome that do not bind to MHC class I molecules do not accumulate in the cell [42], but most of them are rapidly hydrolyzed by downstream proteases and aminopeptidases[49].

MHC I-peptide complex formation.

After proteasomal degradation, the transporter associated with antigen processing (TAP) recognize and translocate minor fractions of approximately 1% to the ER lumen. TAP is an ATP-binding cassette (ABC) transporter that is composed of two half-transporters, TAP1 and TAP2 [50], that are both essential for ATP-dependent peptide translocation into the ER lumen[39]. Both subunits contribute to peptide binding and are able to recruit the chaperon tapasin that stabilize the complex [51]. After transport into the ER lumen, the protein fragments are further trimmed by ER lumen residing proteases for loading onto MHC class I molecules, because many peptides are longer than optimal MHC I binding 8-10 amino acid residues. Peptides that have the correct C-terminal residue for binding MHC class I, but that are too long at the N terminus, can be trimmed by aminopeptidases into mature epitopes. Experiments with proteasomal inhibitors indicated that tripeptidyl peptidase II (TPPII) might be able to compensate for the loss of proteasomes. In addition, it was proposed that TPPII trimming was essential because the proteasome always gave rise to too long peptides. However, a recent study in TPPII-knockdown mice demonstrated TPPII was indeed important (although not essential) for converting long peptides to shorter forms [52].

The peptide processing in the ER lumen is achieved by the aminopeptidase ERAP1 and ERAP2, which are capable of creating and destroying MHC ligands[53]. ERAP1 favors peptides of 9-16 residues and cleaves peptides with a hydrophobic N-terminus. In contrast ERAP2 cleaves peptides with basic residues [54]. The trimmed peptides in length of 8-10 amino acids are rapidly recruited into the peptide loading complex (PLC). The assembly of the PLC occurs in tightly regulated sequential process.

At first, MHC class I molecule its heavy chain is synthesized. The unfolded heavy chain will associate with the transmembrane-bound chaperone calnexin via a monoglucosylated sugar moiety ($Glc_1Man_9GlcNac_2$) that is chemically linked to a conserved asparagines at position 86 of the heavy chain[55]. After binding of calnexin, MHC class I molecule is

deglucosylated by glucosidase II (GII) and reglucosylated by UDPGlc:glycoprotein glucosyltransferase (GT). Calnexin also recruits the ER residing protein ERp57. ERp57 is a member of the protein disulphide isomerase (PDI) family of proteins, which reduce, oxidize or isomerize disulphide bonds[56]. ERp57 is able to form several direct conjugates with the heavy chain via transient disulphide bonds with specific cysteine residues of the heavy chain [57]. Once MHC class I molecules are properly folded, they are no longer recognized as a substrate for GT. However, MHC class I molecules that cannot fold successfully are delivered to the ER-derived quality control compartment (ERQC) and further trimmed to mannose 6 and mannose 5 by ERManI and some accessory mannosidases. This trimming results in recognition by the lectin OS9, which cause translocation to the cytosol, subsequent ubiquitinylation resulting in degradation by the proteasome. This process is called ER-associated degradation (ERAD) [58]. For efficient transport, ubiquitination, and proteasomal degradation, the different proteins form a complex over the ER membrane. Derlin-1 and its homolog, Derlin-2, interact with p97 and its cofactors via the connector protein, VIMP. P97 then directly binds to a ubiquitin-ligase complex that consists of HRD1 and SEL1L. The lectins OS9 and XTP3B associate with BiP and Grp94 in addition to interact with the HRD1/SEL1L complex. The proteasome associates with this complex, allowing this complex to cause retrotranslocation, ubiquitinylation, and subsequent proteasomal degradation of the improperly folded MHC class I molecule [59].

When the MHC class I molecule is properly folded, β2-microglobulin associate with the MHC class I molecule heavy chain. Upon binding of β2-microglobulin, calnexin is replaced by the chaperone calreticulin. This complex associate via tapasin to the TAP complex and forms the peptide loading complex [51]. In the PLC, tapasin acts as a bridge between TAP and the remaining PLC components. Hence, it stabilizes the empty MHC class I complexes and promote binding of high affinity peptides [60]. Peptide binding to the MHC class I protein occurs through the peptide its side chains with pockets in the peptide binding groove of the MHC class I molecule. Only 3 N-terminal and the last C-terminal residues of TAP subunits are involved in substrate recognition, allowing divers substrate binding. This is reflected in the remarkable promiscuity in substrate length by binding peptides of 8-16 residues with equal affinity [61], which matches with the majority of protein fragments after proteasomal degradation. Upon PLC peptide binding, dissociation of the MHC class I molecule-peptide complex is induced in a TAP-signal dependent manner [62].

MHC I transport.

After release of MHC I-peptide complexes from the PLC, the complexes cluster in ER exit sites. MHC class I molecules themselves do not contain sequence motifs for association with the coats of ER export or retrieval vesicles. In addition, peptide-loaded MHC class I molecules were not synchronously released from the ER. These findings imply that MHC class I molecules either leave the ER by bulk flow, or that they are associated with specific carriers. A recent study demonstrates that the transport receptor Bap31, known for transport of membrane proteins from ER to ERGIC, forms oligomers that associate with MHC class I molecules. Bap31 associates with peptide-loaded MHC class I molecules to a greater extent in comparison to nascent MHC class I molecules [63]. Previous reports already demonstrated that Bap31 increases the rate of ER-to-Golgi transport of MHC class I molecules and their stability, and so increases the overall level of surface presented MHC class I-peptide complexes[64]. The overexpression of Bap31-homoloque Bap29 reduces the level of surface expressed MHC class I molecules in an Bap31-Bap29 interaction-dependent manner [64]. However, both overexpression of the negative regulator Bap29 or lack of Bap31 did not abrogate expression of MHC class I molecules, but slowed down the export from the ER [64]. This indicates that redundant mechanisms occur in the investigated cell type.

Eventually, MHC I-peptide complexes are expressed at the plasmamembrane, where they can be recognized by NK cells or cytotoxic $CD8^+$ T cells. To inhibit constitutive antigen presentation, MHC I-peptide complexes are removed from the cell surface by endocytosis. MHC class I molecule lacks sorting signals recognized for endocytosis via clathrin-coated vesicles [65]. However, it has been shown that

MHC I-peptide recycles from endosomes to the cell surface through tubules modulated by Arf6, EHD1, Rab22, and Rabenosyn-5[66-69]. Kirchausen and colleagues observed long and relatively stable tubes containing MHC-I radiating from a perinuclear endosomal compartment towards the cell surface. After increasing the amount of tubules with a HIV-derived mutant protein NefAAA, they were able to demonstrate that the tubules represent deep invaginations of the plasma membrane that remain open to the cell exterior. Live-cell imaging showed that MHC class I molecules can move from the cell surface to endosomes along these tubules, whereas transferrin receptor is mostly excluded. Therefore the authors propose that these tubules might constitute an additional route for MHC-I traffic [70].

§3: Canonical antigen presentation via MHC class I molecules: cross-presentation

As previous chapter points out is antigen presentation of classical MHC I tightly regulated and is in most tissues the presentation in steady state restricted to endogenously generated peptides. The killing by cytotoxic T cells is therefore import to be restricted to only those cells that presents foreign peptides, derived by infection or tumor-associated transformations of self-proteins. However, not all pathogens reach the cytosol. Besides, pathogens compromise the function of APCs. Clearly, this indicates that another mechanism next to the classical pathway of antigen processing via MHC I must operate within APCs. This process is cross-presentation.

Cross-presenting cells.

Cross-presentation is presentation of exogenous, instead of endogenous, peptides via MHC class I molecules to naïve $CD8^+$ T cells and can lead either to the tolerization or activation of antigen-specific $CD8^+$ T cells. At first, in vitro experiments demonstrated that murine lymphoid cells were capable of presenting exogenous antigens present in the culture medium via MHC class I molecules[71]. Later on, *in vivo* experiments demonstrated the presentation of exogenous derived peptides via MHC I [72].

Initially, DCs and macrophages were identified as the immune cells capable of cross-presentation [73,74], but more recently also B-cells [75,76], neutrophils [77,78], and endothelial cells[79] are demonstrated to be capable of cross-presentation under certain conditions. However, DCs are likely the most critical for cross-presentation, because depletion of DCs *in vivo* lead to a lack of CTL responses against cell-associated antigens [80] and it was determined that DCs could cross-present exogenous antigens on MHC class I molecules as effectively as presentation via MHC class II molecules in a quantitative measurement [81]. In addition, immature DCs actively control alkalinization of their phagosomes [82], have low lysosomal proteolysis [83], and express protease inhibitors [84], thereby increasing the chance that exogenous antigens in the phagosome are cross-presented [83]. This is supported by the finding that exogenous soluble OVA was cross-presented by immature DCs [85].

Experiments done in mice infected with viruses [86], or immunized with cell-associated or soluble antigen [87,88], indicate that $CD8\alpha^+$ DCs are the primary APC responsible for cross-presenting antigen to naive $CD8^+$ T lymphocytes. However, cross-presentation is not restricted to the $CD8^+$ DCs as illustrated by presentation by $CD8\alpha^-$ DCs of antigenic peptides derived from immune complexes[89], suggesting that $CD8\alpha^+$ DCs possess specialized machinery required for effective cross-presentation [90].

$CD8^+$ DCs are thought to occur mainly in secondary lymphoid tissue [91] and absent from antigen-capturing sites such as the skin [92] therefore it is hypothesized that tissue-derived $CD8\alpha^-$ DCs may transport antigen to draining lymph nodes and transfer it to lymphoid resident $CD8\alpha^+$ DCs for cross-presentation and priming of naïve T lymphocytes [93]. To date, mechanisms for inter-DC antigen transfer remain unsolved question. It is proposed that exosomes released by migrating DC could serve as antigen spreaders within the lymph nodes [94], whilst others suggested transfer via apoptotic bodies [95]. Probably this inter-DC antigen transfer serves to amplify antigen presentation across a larger network of lymphoid-resident DCs for efficient T cell activation. This model is supported by recent reports that demonstrate that specific protein antigens are conserved in intracellular storage depots for many days [96].

Antigen processing.

To be able to present the antigen via MHC class I molecules, the antigen requires proteolytical processing into the appropriate length for binding into the peptide-binding groove of the MHC I molecule. Since the proteasome is demonstrated to be the primary source of peptides in the classical MHC I pathway, and logically APCs prime naïve $CD8^+$ T cells with similar peptides as present on infected cells, it is not unexpected that proteasome activity was reported to be essential for cross-presentation[97,98]. However, many other reports have demonstrated contradicting data that show proteasome-independent processing of the exogenous protein for cross-presentation via MHC class I molecules[99,100] in the vesicular compartment via endo/lysosomal proteases, in particular cathepsin S[100]. This controversy has led to two different models, the cytosolic pathway and vacuolar pathway.

The cytosolic pathway proposes that the antigen is transported after internalization into the cytosol of the APCs. Subsequently, the antigens are degraded by the proteasome, before being transported to the location where newly synthesized MHC class I molecules are loaded with the peptides. This is supported by reports demonstrating that peptides were generated in non-lysosomal compartments since inhibition of endo- and lysosomal acidification by chloroquine enhanced cross-presentation [101]. The cytosolic pathway was proved with the use of gelonin. Gelonin is membrane impermeable and nontoxic to intact cells, but inhibits protein synthesis when it gains access to the cytosol. Gelonin coupled to beads was demonstrated to significantly inhibit protein synthesis in macrophages. To mediate its toxic effect in macrophages, gelonin must have been transferred intact from the phagosome into the cytosol.

Since these exogenous antigens access the cytosol from the vesicular compartment and ultimately use the classical MHC class I presentation pathway, this process depends on the proteasome, as demonstrated by using proteasome inhibitors [97].

Exogenous antigens can be internalized by APCs in different ways, respectively soluble proteins are internalized by endocytosis or macropinocytosis, whereas particulate antigens are internalized via phagocytosis. The primary source of antigens for cross-presentation seems to be phagocytosis, because particulate antigens are more efficiently targeted for cross-presentation in comparison to when they are soluble. This is demonstrated by a lack of CD8 T cell response after immunization with soluble OVA, whilst immunization with latex bead-coupled OVA elicited an OVA-specific CD8 T cell response in vivo[102]. In addition, inhibition of phagocytosis by administration of cytochalasin B resulted in lack of antigen presentation[103]. For generation of peptides via the proteasome, the antigen must migrate from the phagosome to the cytosol.

It is proposed that this occurs via a selective and size-specific process that permits transfer over the phagosomal membrane [104]. Proposed mechanisms is the ERAD translocon sec61, which inhibition with exotoxin A of *Pseudomonas aeruginosa* resulted in lack of cross-presentation [105]. After degradation in the cytosol the generated peptides are via TAP transferred into the ER lumen, supported by the fact that TAP-deficient mice APCs are unable to cross present [97]. Inside the ER lumen, the peptides intersect with the classical MHC class I antigen presentation pathway, as indicated by results obtained with Brefeldin A. Brefeldin A is an inhibitor of protein transport from ER to Golgi and was demonstrated to inhibited cross-presentation in certain conditions [97,106].

The recent established role of the GTPase Irgm3 in cross-presentation is perhaps supporting this model. Since Irgm3 deficiency did inhibit cross-presentation, but no the loading of exogenous peptides on MHC class II molecules in the phagosomal lumen, suggests that Irgm3 might play a role in the translocation step into the cytosol [107]. This is supported by the fact that Irgm3-deficient mice elicit an immune response against peptides that are delivered directly in the cytosol[107]. Irgm3 associates with lipid bodies (LBs). These LBs have a monolayer membrane, thus it is possible that these LBs interact with the phagosomal membrane thereby influencing the lipid organization and/or association of cytoplasmic anchored proteins including Irgm3.

Another cytosolic pathway is proposed in which endosomes fuse with the ER. This because soluble antigens were found to

associate with ERAD pathway components in the ER after internalization and cross-presentation was inhibited when ERAD component levels were reduced by silencing RNA's[108]. However, since it was showed that the cross-presentation of particulate antigens via phagocytosis was more efficiently than endocytosed soluble antigens the physiological relevance is doubted.

However, the finding that ER-resident proteins could be found within phagosomes gave rise to another model[109]. This model proposes that phagosomes in macrophages and DCs are competent organelles for cross-presentation[51,110,111]. It was demonstrated that macropinosomes fuse with the ER in human DCs, so they contained many components of the retrograde translocation machinery. This ready-made export to cytosol complex would explain the rapid export of phagocytosed antigens into the cytosol within 1-2 h after internalization. Together with the finding that both TAP and MHC class I molecules are present in these ER-fused phagosomes, this indicate an autonomous compartment[110,111]. This is supported by the fact that this process is partially inhibited by Brefeldin A[112]. In addition, recent studies demonstrate the existence of an endosomal storage compartment in which antigens can be stored for a while and are not delivered to lysosomes[113,114]. Recent report demonstrate that the ubiquitous zinc-dependent aminopeptidase IRAP, which is closely related to ERAP1 and ERAP2, is present in these Rab14^{+} endosomal compartments. Deficiency of IRAP compromised cross-presentation that was proteasome- and TAP-dependent, but is not lysosomal protease dependent[113]. As proposed, it was recently demonstrated that phagosomal TAP relocates cytosolic antigenic peptides back into the early stable endosomes, where peptide is loaded onto MHC class I molecules and transported to the plasmamembrane for antigen presentation[115].

This autonomous compartment can hypothetically contribute to a rapid cross-presentation response by efficiently bringing all necessary components in proximity of one compartment. In addition, the spatial separation of MHC class I–restricted antigen processing for endogenous and extracellular antigens may ensure that peptides from extracellular antigens do not have to compete with the large pool of endogenous peptides for MHC class I molecules in the endoplasmic reticulum[115].

It is doubtful that the ER contribute to the phagosomal membrane as only a small part was demonstrated to contribute to them [116] and mathematical calculations predicted that cross-presentation via the ER-phagosome pathway would be highly inefficient[117]. Therefore, some sort of delivery vesicle is likely to be required in this process.

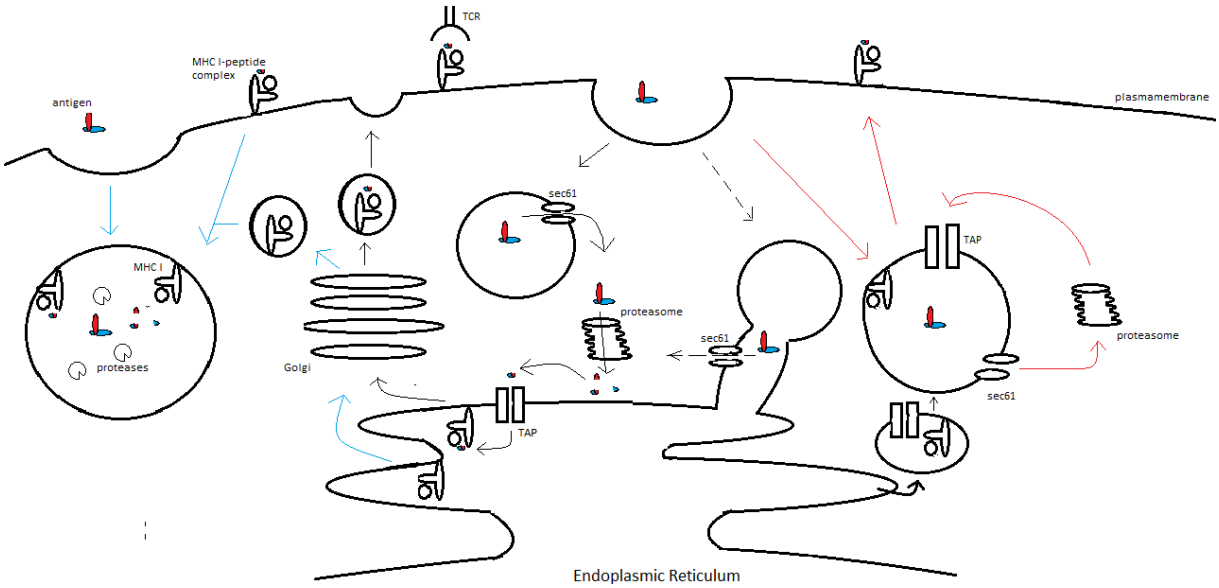


Fig.1. schematic depiction of all proposed pathways of cross-presentation. Blue arrows point out vacuolar pathway, whereas the black arrows indicate the Sec61-, TAP-dependent, BFA-sensitive pathways. The red arrows route the Sec61-, TAP-dependent, partially BFA-sensitive pathway.

In contrast of the cytosolic pathways, also another pathway is reported. This alternative pathway is independent of both TAP and proteasome, and could not be inhibited by Brefeldin A[99,100,118-124]. In this model, proteins are degraded by proteolytic processing by lysosomal proteases and the generated peptides are loaded onto MHC class I molecules inside the vacuolar compartment and subsequently transferred to the cell surface for presentation. Shen *et al* [100]demonstrated that OVA was cross-presented in a TAP-dependent as well as TAP-independent pathway, and that the TAP independent cross-presentation of OVA by DCs required cathepsin S, but no other cathepsins. This was also demonstrated *in vivo* when TAP-deficient mice with OVA incorporated into microspheres of biodegradable co-polymer polylactide polyglycolide (PLGA-OVA) elicit a proliferative OVA-specific $CD8^+$ T cell response, in contrast to TAP/cathepsin S-double deficient mice. This model is also supported by finding of the long lasting vesicular compartment[113,114].

The main question in this model was whether MHC class I molecules in this pathway are derived from the ER or if they are recycling MHC class I molecules from the plasmamembrane. Since only peptide-bound MHC class I molecules are transported to the plasmamembrane, peptide-exchange must be able to occur. *In vitro* experiments indicate that MHC class I complexes can retain their integrity until pH 5.5, but at pH 5.0 the MHC class I complex first releases its peptide and at pH lower than 5.0 the heavy chain-β2m heterodimer dissociates completely. These results suggest that at pH 5.0, which corresponds to the pH of late endosomal/early lysosomal compartments as the MIIC, peptide-receptive “empty” MHC class I complexes can be generated[125]. However, many still question the logic of this pathway. At first, lysosomal proteolysis cannot reproducibly generate similar peptides as the cytosolic pathway. Secondly, the interaction between MHC class I molecule and antigen peptides is destabilized by the low pH, is a stable association between other antigenic peptides with MHC class I molecules possible.

As indicated by Shen *et al*[100], multiple pathways mentioned above can coexist in the same cell type. This is possible since most of these pathways are compartmentalized and are strictly regulate. Since the cross-presentation is a continuous membrane transport process that begins from the endocytosis of an exogenous antigen and ends with the expression of MHC class I molecule-peptide complexes on the cell surface, a major role is played by Rab GTPases that are involved in regulation of vesicle trafficking. Rab GTPases represent a large family of proteins that are recognized as key regulators of membrane trafficking. Recruitment of Rab14 may reduce routing of antigens into an acid lysosomal environment known to be detrimental for cross-presentation [113], thereby giving rise to the stable storage compartments. In contrast, Rab3b/3c are involved in transport to the plasmamembrane of vesicles containing MHC class I-peptide complexes that were formed in the storage compartment [126]. Also other Rab GTPases are demonstrated to play a role in cross-presentation, such as Rab4a, Rab5b, Rab6, Rab8b, Rab10, Rab27a, Rab32, Rab33a, Rab34, and Rab35[126]. For instance, Rab27a is important to generate a phagosome dedicated to cross-presentation and is specifically recruited to phagosomes facilitated by immunoglobulin binding[127].

This compartmentalization requires sorting and specific targeting to compartments of the antigens.

Contribution to antigen presentation of bacterial-derived carbohydrates as the context of antigens.

A hallmark of the adaptive T cell response is the ability of T cell receptors (TCR) to specifically recognize a vast array of different antigens. However, while this recognition plays a role in initiating T cell activation, the precise nature of the response is dictated by environmental cues within the inflammatory milieu. The outcome of antigen recognition is not determined by the nature of the antigen itself, but rather by the context in which the antigen is encountered. The cell wall of microbes are decorated with a wide array of carbohydrates, such as extracellular and capsular polysaccharides, glycoproteins, and glycolipids, which are predominantly present as context of an antigen.
Initially in this chapter the variety of bacterial carbohydrate structures and the mechanism of recognition by certain receptors will be discussed. Followed by a discussion on the positive contribution of bacterial carbohydrates to the process of antigen presentation.

§1: Carbohydrate structures present on bacteria

In epidemics or local outbreaks of a certain disease, it is important to monitor the spread of the causing agent and serotyping, if possible, is the simplest tool. The serogrouping/serotyping is based on the reactivity of specific antibodies using reference strains of particular species, with the microorganism. The specific antibodies are usually directed against the surface carbohydrate antigens. The species *S. pneumoniae* is divided into more than 90 serotypes based on the carbohydrate structure [128], reflecting the structural diversity of the polysaccharides. Thus bacteria express a large variety of extracellular carbohydrate structures. Some excrete large-molecular-weight polysaccharides that retain only limited association with the bacterial surface, and these are often referred to as extracellular polysaccharides (EPS) or slime polysaccharides. In contrast, other bacteria form a discrete surface layer of these polysaccharides that is intimately associated with the cell surface, and is called the capsule. These large polymers are important virulence determinants, as enzymic depolymerization of the capsular polysaccharide on the surface of *S. pneumoniae* type 3 was found to decrease considerably the virulence of the microbe. The main mechanism of virulence is that polysaccharides inhibit the rapid alternate pathway of complement activation to oblige the host immune system to use the slower classic pathway of compliment activation[129]. This phenomena occurs by several mechanisms: the large polymers can mask underlying molecules that could activate the complement system, sialic acid containing polysaccharides favor binding of serum proteins that specifically inhibit amplification of the alternate pathway, and amplification of C3b deposition is decreased by less binding affinity of factor B for capsulated bacteria [130].
Other carbohydrate structures present in bacteria and amongst others are the glycans, including glylipids and glycoproteins. Most organisms glycosylate as a post-translational modification an extensive part of their surface-expressed and secreted proteins, which is important in stability of the proteins, protection against protealytic cleavage and adhesion [131]. The diversity of glycans exceeds the diversity of proteins and nucleic acids as a result of the different disaccharide linkages, anomerization, braching, and modifications of hydroxyl-groups of saccharides by various groups[132]. Based on the link between saccharide and protein or lipid, the glycans are divided in three major classes: N-linked, O-linked, and glycosylphosphatidylinositol (GPI)-anchored glycans.
N-linked glycans are the most abundant class that consists of a pentasaccharide core that can be extended by up to 5 branches of other saccharides. This oligosaccharide is attached to proteins via an asparagine residue embedded in the Asn-X-Ser/Thr consensus sequence, with X for every possible amino acid. In eukaryotes further modification of the N-glycan occurs in the ER and Golgi-complex, where it plays an essential role in the quality control system of protein synthesis. In general, lower eukaryotes predominantly contain polysaccharides that are modified into high-mannose structures, while higher eukaryotes produce more complex glycans[133].

In contrast to N-linked glycans does O-linked glycans do not contain a common core structure and are less abundantly expressed. The polysaccharide is covalently attached to the amino acid side chains via hydroxyl group of serine or threonine residues. In eukaryotes, the O-glycosylation occurs on properly folded proteins in the Golgi complex. Bacteria contain a relatively large amount of O-linked glycoconjugates, while human O-glycans are mainly restricted to mucins and collagens.
The last class in glycans is the GPI-anchored glycans. These GPI-anchors attach proteins or glycoproteins to eukaryotic cell membranes and are naturally occurring glycolipids. Only a few GPI-anchored glycans are discovered in microbes.
To manage antigenic-diversity and immune evasion, bacteria express a unique set of glycosyltransferases from phase-variable genes [131]. The glycosyltransferases are enzymes that are able to catalyze the stepwise synthesis of both N- and O-linked polysaccharides[134].

§2: Recognition of bacteria via their carbohydrate structures.

The innate immune response involves binding of soluble and membrane bound host molecules to the surface of pathogenic organisms including viruses, bacteria, parasites and fungi. The dogma until recently was that these pathogen-associated molecular patterns (PAMPs) are unique for the pathogen, restricting the immune response to non-self antigens. Hence, the germline-encoded receptors recognizing the molecular patterns are named Pathogen-recognition receptors (PRRs). However, recent research on lectins has identified multiple epitopes recognized on pathogens that are also present in the host and involved in cellular processes [135]. Hence, the molecular basis for pattern recognition by lectins of carbohydrate epitopes on pathogens is in question.

Recognition of carbohydrate structures by Lectins.

Lectins are host molecules involved in glycan binding by interaction with their carbohydrate recognition domains (CRDs). These CRDs are able to selectively recognize complex oligosaccharides, determined at first by the amino acid sequence comprising the CRD [136]. The classical CRDs can be subdivided in two broad groups, mannose and galactose-binding types. The mannose-binding CRDs contain the triplet amino acid sequence EPN, whereas the galactose-binding CRDs contain the QPD triplet [137]. However, only one or two residues determine the recognition and appear to act as anchors driving the glycan-protein interaction. Using so few residues in recognition allows for less restricted pool of ligands, as indicated by an overlapping array of ligands that contribute to redundancy.
The glycan-protein binding is determined by different factors; hydrogen-bonding, which is significantly reduced by competition from bulk solvent and by the flexible nature of the hydroxyl groups; ionic interaction; and stacking interactions by sugar CH-bonds with protein aromatic side chains. Since natural carbohydrates usually lack extended hydrophobic areas, the later indicated that the affinity of lectins for monovalent carbohydrates is typically weak. Indeed, lectins have dissociation constants that are in the range of mM to μM. In the specific case of C-type lectins, the lectin-carbohydrate interaction by the CRD also depends on ionic interactions with a calcium ion and thus reacts in a calcium-dependent manner. Hence the name C-type lectins.
Recent data of quantitative studies show that lectin affinity to glycans is sensitive to glycan density and number of glycan epitopes. Data demonstrates that the binding of lectins to high density and number of glycans can lead to threshold binding effects (Orr, G., A., Rando, R.R., et al. 1979), very large increases in affinity ($\sim 10^6$-fold) [138] and cross-linking interactions [139], which can result in a variety of “effector” functions in the host[140,141] as well as pathogenic organism[142,143]. Two mechanisms are identified that are central to the density dependent binding of lectins: binding and internal diffusion of lectins among a dense population of glycans [144], and binding of multiple CRD domains of lectins to clustered glycan epitopes [145], respectively. The latter mechanism is likely to utilize the first mechanism as well.
The glycan density dependent lectin binding indicates that the “pattern” that will be recognized by host lectins is the high density expression of a glycan epitope on the surface of host cells and foreign pathogens. In many cases, the glycan epitope expressed on the foreign pathogen is a low affinity epitope not found on the host. However, host lectins may bind to such “weak epitopes” due to large

increases in the affinity of the lectin for polyvalent displays of the glycan by either one of the two mechanisms discussed above.
In addition to density dependent glycan expression, the "threshold" for glycan recognition is also a function of the concentration of the lectin in solution[146]. Thus both density dependent glycan and lectin expression are important determinants of the innate immune response and host glycan-lectin interactions. Hypothetically, this also suggests that essentially all lectins, whether from animals, plants, or microorganisms, are PRRs, where pattern (often mentioned as "the glyco-code") is defined by the density and total number of glycan epitopes on glycoprotein receptors on the surface of cells[139].

Recognition of carbohydrate structures by antibodies.
Specific antibody diversity is generated in an antigen-independent fashion during the differentiation of B lymphocytes by a number of mechanisms including recombination of multiple gene segments, combinatorial diversity of both chains of the immunoglobulin, somatic hypermutation, and gene conversion. The result of these genetic events is the generation of B lymphocytes with surface membrane immunoglobulin molecules that accommodate an enormous number of potential antigens, leading after antigen-specific recognition to B cell proliferation, differentiation, and the ultimate secretion of large amounts of antigen-specific antibodies [3]. In general, anti-carbohydrate antibodies are known to display low affinities for simple monosaccharides [147,148], as similar to lectins. Lectins their determinants often require multiple carbohydrate residues to reach the degree of affinity needed for specific recognition. Additionally in comparison to lectins, structural and kinetic data demonstrated that ligand binding by antibodies is not a static but highly dynamic process and conformational changes can occur upon ligand binding. It was demonstrated that antibodies can exhibit conformational diversity by adopting multiple free structures. Hence, antibodies are capable of binding unrelated antigens[149].

The precise carbohydrate structure is essential, since the slightest structural differences in polysaccharides can give rise to distinct immune responses or immune recognition. An example are CPSs of Group B streptococcus type Ia and Ib, which have both five identical sugars in its repeating units that are all linked identically except for one β1→4 versus β1→3 linkage between Gal*p* and GlcNAc, and leads to distinct antibody responses that are not cross-protective [150].

Carbohydrate-receptors.
Lectins.
Carbohydrates can be recognized by a variety of PRRs. The already mentioned lectins are a major class that is able to recognize primarily glycans via the CRDs. This family is composed of three major classes; C-type lectins, sialic acid-binding immunoglobulin-like lectins (siglecs), and galectins. These members contain one or more CRDs and exist either as transmembrane domain or soluble proteins. Transmembrane CLRs can be divided into two types. Type 1 CLRs comprise the Mannose Receptor (MR) and DEC-205, and contain several CRDs or CRD-like domains and have an extracellular N-terminus. In contrast, type II CLRs comprise DC-SIGN, Langerin, DCIR, CLEC-1, DLEC, BDCA-2, Dectin 1, and Dectin 2. They have only one CRD and have an intracellular N-terminus. CLRs contain a hallmark C-type lectin fold consisting two anti-parallel β strands and two α helices. This prototypic fold contains irregular loop structures from which two are involved in monosaccharide binding[151].
Soluble C-type lectins are known as collectins, including Mannose-binding lectin (MBL), surfactant protein A (SP-A), and surfactant protein D (SP-D). Their CRDs are found in associateion with collagenous structures, hence the name[152].
Another family that is able to recognize carbohydrate structures are the pentraxins, subdivided in short pentraxins composed of C-reactive protein (CRP) and serum amyloid P component (SAP), and long pentraxins that include pentraxin 3 (PTX3). The Pentraxins are evolutionarily conserved proteins, characterized by a cyclic multimeric structura and the 200 amino acid pentraxin domain (HxCxS/TWxS) with an 8 amino acid-long conserved pentraxin signature in their C-terminus. A list of selected ligands recognizing pentraxins is provided in table 1. However, the relationship between ligand binding and function of pentraxins is still under debate [153]. The short pentraxins are soluble and

secreted from the liver into the blood stream, in which they are able to recognize different ligans, such as damaged cells or bacteria. CRP and SAP aggregate or attach to most of their ligands, which are subsequently recognized by Fcγ receptors and complement components[154].

Three ficolins are identified in humans: L-, H-, and M-ficolin. In general, they have an N-terminal region, a collagen-like domain that is involved in activation of the lectin-complement pathway, and a C-terminal protein that is homologous to the fibrinogen β and γ chains [155]. This fibrinogen-like domain is structurally and functionally similar to CRDs, because it is also involved in recognition of microbial moieties and have a globular structure. Two murine ficolins are described: ficolin A that is homologous to L-ficolin, and ficolin B that is homologous to M-ficolin [156]. Ficolins are characterized by a specificity for N-acetyl glucosamine residues in complex-type oligosaccharides, but not for mannose or high-mannose type oligosaccharides[155]. In addition, H-ficolin binds GalNAc and D-fucose residues, whereas M-ficolin binds sialic acid[157].

Toll-like receptors.

Toll-like rceptors (TLRs) are type I transmembrane molecules with an extracellular domain containing leucine-rich repeats and cytoplasmic TIR domain (Toll/IL-1 receptor), homologous to the IL-1 receptor. They were first discovered in Drosophila melanogaster as molecules required for dorso-ventral patterning in embryogenesis[158]. It was then realized that some members of the Toll receptor family also played a role in immunity in insects, because mutations in toll genes made flies more susceptible to infections[159]. Discovery of Drosophila Tolls led to the discovery and cloning of human TLRs[160]. Humans have 10 TLR receptors, and different TLRs are specific for different microbial products [161]. Different TLRs can form homo- or heterodimers.

The cell-activating function of TLR2 requires the formation of heterodimers with TLR1 or TLR6 [161]. TLR2 functions as a cell-activating receptor for peptidoglycan, lipoteichoic acid, lipoproteins, lipopeptides, mycobacterial lipoarabinomannan, and fungal cell walls (zymosan)[161]. TLR2 may even function as a receptor for glycolipids, glycoinositol-phospholipids and necrotic cells [162,163].

Peptidoglycan is an essential cell wall component of virtually all bacteria[164]. PGN is a polymer of b(1–4)-linked N-acetylglucosamine and N-acetylmuramic acid, crosslinked by short peptides (fig. 1) [164]. The glycan chain is usually N-acetylated and sometimes O-acetylated, and is relatively similar in all bacteria. PGN is an excellent target for recognition by the eukaryotic innate immune system because PGN is an essential and unique cell wall component of virtually all bacteria, and because it is not present in eukaryotic cells [164].

Recent studies suggest that peptidoglycan directly binds to TLR2[165]. Cell activation studies indicate that a sequence of 25 amino acids (Ser40–Ile64) in the extracellular domain of TLR2 is required for the peptidoglycan-induced cell activation[166], thus suggesting that this sequence may constitute (or be a part of) the TLR2 binding site for PGN. The exact PGN structure recognized by TLR2 is not known.

TLR4 in company with its coreceptor myeloid differentiation factor 2 (MD-2) is specific for recognition of lipopolysaccharides [167,168]. A recently discovered soluble form of MD-2 is also shown to function as an opsonin in a TLR4-dependent pathway [169].

Lipopolysaccharides (LPSs) are outer-membrane glycolipids of Gram-negative bacteria and are well-known inducers of the innate immune response[170]. They are composed of a hydrophobic lipid A component and a hydrophilic polysaccharide component. The lipid A portion is composed of phosphorylated diglucosamine and four to seven acyl chains. Both the 1 and 4′ positions of the glucosamine backbone of lipid A are frequently phosphorylated. Phosphorylation is important for the biological activity of LPS because diphosphorylated lipid A is more than 1000-fold more active than monophosphorylated lipid A[171]. The phosphate group can be further modified by other chemical groups. Four to seven mostly saturated lipid chains are linked to the sugar backbone of lipid A through either ester or amide linkages. The lipid A moiety is connected to the core oligosaccharide part of LPS. The core sugar chain contains unusual Kdo (3-deoxy-D-manno-oct-2-ulosonic acid)

and heptose saccharides not normally found in humans and is connected to the highly variable O-specific chain composed of repeating oligosaccharide units[170]. The O-specific sugar chains are highly variable in structure compared to the core sugar. The number of repeating units can be anywhere from 1–50 in the same bacterium. The sugar composition, sequence, chemical linkage, substitution, and ring form of the repeating units can vary dramatically, thereby making LPS of almost unlimited diversity. Experiments using synthetic variants of lipid A demonstrate that lipid A containing structural changes in the sugar or lipid chains invariably lead to substantially reduced activity [172]. MD-2 adopts a β cup fold with two antiparallel β sheets that are separated on one side, and with internal hydrophobic residues exposed for ligand binding[173]. The internal pocket of MD-2 is completely lined with hydrophobic residues, but the open region has positively charged residues. Therefore, the overall shape and electrostatic characteristics of MD-2 seem to be suitable for binding negatively charged amphipathic ligands such as LPS. MD-2 is the only LPS-binding component of the TLR4-MD-2 complex[174].

Table 1. Summary of receptors for bacterial-derived carbohydrate structures.

Receptor	Expression	Foreign ligands	Reference
MR CD206	Macrophage, moDCs, lymphatic and hepatic epithelium, kidney mesangial cells, tracheal SMCs, and retinal pigment epithelium	Mannose, fucose or GlcNAc sugar residues of: *Candida Albincans, Pneumocystic carinii, Leishmania donovani, Mycobacterium Tuberculosis*, CPS of *Klebsiella pneumoniae, Streptococcus pneumoniae*	[175-180]
Dectin-1	Widely expressed, including macrophages, dcs monocytes, neutrophils, and splenic T cell subset. (human dectin-1 also expressed on B cells, eo's, and mast cells	β-glucans, found on mainly cell walls of funghi, but also in plants and some bacteria.	
DC-SIGN	ImmDCs, endothelium, macrophage subpopulations	Internal mannose branched structures, terminal dimannose, favor high mannose polysaccharides ICAM3 *M. tuberculosis, C. albicans, H. pylori, Schistoma mansoni, A. fumigatus*.	[181-192]
MBL	Secreted into blood stream, primarily produced by liver, but also intestine.	Mannose, glucose, l-fucose, ManNAc, GlcNAc of: *E.coli, Klebisella aerogenes, Neisseria meningitides, S. aureus, S. pneumoniae, A. fumigators, C. albicans.*	[193-196]
Surfactant A and D	Type II and Clara cells	SP-A> l-fucose and N-acetylmannosamine (ManNAc) SP-D > glucose, maltose, and inositol. *Pseudomonas aeruginosa, P. carinii, A. fumigatus, M. tuberculosis, S. pneumoniae, K. pneumonia*	[197-204]
C-reactive protein	Hepatocytes	Capsular PS of *Streptococcus pneumoniae, Klebsiella aerogenes*	[205]
Serum amyloid P component	Hepatocytes	*Streptococcus pyogenes, neisseria mengitidis*, LPS of *E. coli, S. typhimurium*	[206]
Ficolins	Hepatocytes, type II alveolar cells, PMN, monocytes	L-ficolin: GclNAx, complex-type oligosaccharides with GlcNAc residues linked to trimannosyl core. H-ficolin: GlcNAc and GalNAc, fucose LPS of *Salmonella typhimurium, Salmonella minnesota* and *E. Coli* (O111). M-ficolin: sialic acid residues, GlcNAc, LagNAc, sialyl LacNAc	[156,157,207,208]
TLR2	microglia, Schwann cells, monocytes, macrophages, dendritic cells, polymorphonuclear leukocytes, B cells, and T cells	lipoteichoic acid, peptidoglycan, lipoproteins (MALP-2, MALP-404), lipomannan, zymosan, Lipophosphoglycan	
TLR4	Schwann cells, monocytes, macrophages, dendritic cells, polymorphonuclear leukocytes, B cells, and T cells	Lipopolysaccharide, heat shock proteins, fibrinogen, heparin sulphate, hyaluronic acid	

§3: Consequences of extracellular recognition of bacterial-derived carbohydrates on bacterial uptake.

Collectins, short pentraxins, and polysaccharide-specific antibodies are soluble factors that are able to bind to the surface of bacteria by recognition of bacterial carbohydrate structures, e.g. opsonize the bacteria. Opsonized bacteria are dependent on the opsonin recognized by either Fcγ receptors (FcγR), and/or complement components leading to recognition by complement receptors (CRs)[154].

In the case of the short pentraxins, a specific and saturable binding to all three classes of FcγRs has been demonstrated. The crystal structure of human SAP in on complex with FcγRIIa indicates that the ridge helices of pentameric SAP interact with the FcγR [209].
The MBL and other collectins interact with the phagocytic receptors C1qRp (CD93) and cC1qR (calreticulin). SP-A and SP-D recognize bacteria with their CRD, whilst the free collagen tail binds to the cellular receptors. MBL is also recognized by CR1 [210], whereas the surfactant proteins are able to directly bind CD14 thus modulating LPS, TLR 2 and 4 signaling [211].
Bacteria opsonized by IgG antibodies are recognized by Fc receptors, including FcγRs and the MHC class I-related FcRn, important for efficient clearance of the pathogens.
Deficiencies in opsonins or other PRRs show that they are essential in the barrier against pathogens, as indicated by the susceptibility towards varies pathogens[212-214].

Fcγ Receptor-mediated phagocytosis.

The three classes of human FcRs are members of the immunoglobulin superfamily. Upon ligand binding, they signal via immunoreceptor tyrosine-based activation motifs (ITAMs) that conform to the consensus YxxLx5-12Yx-2-3L/I. These motifs are located either in the cytosolic domains of the receptors themselves (FcγRIIA), or within γ associated (FcγRI and FcγRIIIA) or ζ (FcγRIIIA) subunits. Upon ligand binding, FcγRs and associated γ chain cluster together and tyrosine residues embedded in the ITAMs become phosphorylated. The phosphorylation is mediated by Src-family tyrosine kinase [215] that seems to facilitate clustering of the receptors [216]. The phosphorylated tyrosine-residues in the ITAM-domain act as docking sites for Src homology 2 (SH2) domain-containing proteins. Most SH2 domain-containing proteins act as scaffold proteins that function as docking platform for other essential. Critical family in regulation of actin reorganization during a variety of phagocytic signaling processes is the Rho GTP-binding proteins. When activated and bound to GTP, they interact with downstream effectors, whereas inactive GDP-bound Rhofamily G proteins are thought to be cytosolic and bound to a guanine dissociation inhibitor (GDI) [217]. Rho GTP-binding proteins are controlling actin remodeling in phagocytosis by locally recruiting and interacting with a number of downstream effectors in their activated form. In turn, the downstream effectors (such as Wiskott-Aldrich Syndrome Protein and Suppressor of cAMP receptor/WASP family Verprolin-homologous) are then able to bind and activate actin-nucleating proteins, in particular the Arp2/3 complex. This complex functions by promoting de novo actin polymerisation through stimulating the branching of new filaments on existing actin filaments [218]. In FcγR-mediated phagocytosis, Rac1, Rac2 and CDC42 are the major Rho GTP-binding proteins that are effecting actin remodeling [219,220]. Accumulation of active Cdc42 to forming phagosomes occurs early and is preferentially restricted to the tips of extending pseudopodia [221]. In contrast, activation and localization of Rac1 occurs throughout the phagocytic cup and during closure [221]. Local Rac2 activity precedes Rac1 activation, coinciding with particle contraction and phagosome closure [221]. The actin dynamics are not restricted to nucleation and polymerization but are also regulated by capping/uncapping, depolymerisation and severing [222].

CR-mediated phagocytosis.

As indicated by distinct morphologic processes, respectively actin-rich pseudopodia extending circumferentially around tight-fitting (e.g. zippered) phagosomes in the case of FcγR-mediated phagocytosis in comparison to spacious phagosomes that appear to sink into the cell in the case of CR-mediated phagocytosis, CR and FcγR-mediated phagocytosis differ in some respects [223,224]. This polar manner of phagocytosis by CR is

also indicated by the focused localization of downstream effectors just underneath the particulate ligand in comparison to a more dispersed localization of the same effectors upon Fcγ-ligand interaction [225].
In contrast to FcγR-dependent uptake, CR-dependent binding and phagocytosis of opsonised particles is not constitutively active and requires pre-activation of phagocytes [226]. The small G protein Rap1 is sufficient to activate CR, promoting binding and phagocytosis of opsonised targets. In addition, Rap1 is activated in response to inflammatory mediators and its activity is required for agonist-induced activation of CR [227]. Unlike the FcγR, CR does not rely on tyrosine phosphorylation to induce phagocytosis in macrophages, providing evidence for receptor-specific pathways in the early phases of uptake.
In contrast to FcγR-dependent phagocytosis in which actin remodelling is dominated by Cdc42 and Rac, the exclusively activity of another Rho-family protein, RhoA, is essential. Both Cdc42 and Rac are not critical for CR-mediated phagocytosis [219]. Recruitment of Rho to forming CR3 phagosomes has been demonstrated [228].
Another difference between CR- and FcγR-mediated phagocytosis is the F-actin assembly. FcγR-mediated phagocytosis shows continuous F-actin cups, whereas the phagocytic cup during CR-mediated uptake consists of discrete foci of F-actin and other cytoskeletal-associated proteins [219,225]. These downstream effectors are present in almost all cells, even in non-phagocytic cells, implying that only expression of phagocytosis-initiator molecules are lacking in these cells. Indeed, transfection of the FcγR into non-phagocytic human lung epithelial cells transfers the ability to bind and phagocytose opsonized particulate antigens [229].

Lectin-mediated internalization.

Mannose-receptor:

The Mannose Receptor (MR) was implied in bacterial uptake, since transfection with MR into non-phagocytic COS-1 cells led to phagocyt osis of C.albicans and P.carinii [176]. Also phagocytosis of non-opsonized *M. tuberculosis* is mediated by the Mannose receptor, dependent on interaction between MR and the mannose cap of the mannosylated lipoarabinomannan (ManLAM) [179,230,231]. Although the interaction between MR and *M. tuberculosis* is associated with an anti-inflammatory immunosuppressive program, favoring bacterial infection [232].
MR as a phagocytic receptor is supported by a study in which MR-positive macrophages ingested threefold more *Francisella tularensis* in comparison with MR-negative macrophage and MR-specific blocking antibodies reduced level of phagocytosis [233]. As expected, the cytoplasmic tail in which the diaromatic amino acid sequence is embedded is necessary for signaling. However, the tyrosine in this motif is not essential for phagocytosis [234]. Further research have indicated a molecular mechanism that appears distinct of opsonin-dependent phagocytosis, requiring Cdc42 and Rho activation, promoting PAK1 activation, and requiring focal F-actin polymerization and the Rho effector molecule ROCK instead of Rac [235].
However, the fact that a number of different cell lines that express MR are not able to internalize well-known ligands for MR challenge the idea of MR as phagocytic receptor. Therefore, Le Cabec *et al* propose that MR is a binding receptor that needs a partner to trigger phagocytosis[236].

Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin:

Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN) is known to bind ICAM-3 that function as co-stimulatory molecule during T cell stimulation. Nowadays, DC-SIGN research was focused on its role in HIV-1 infection, because HIV-1 is able to bind this molecule [181]. In its cytoplasmic tail, multiple internalization motifs are present, ITAM motif, tri-acidic cluster, and a di-leucine motif, respectively [237]. DC-SIGN as a phagocytic receptor is supported by multiple reports that demonstrated co-localization in vesicles containing A.fumigatus [190], *M. tuberculosis* [191], and C.albicans in a mannan-dependent manner [188]. Transfecting DC-SIGN into HEK293 cells, transfer the ability to phagocytose. Ca(2+) binding sites in the CRD of DC-SIGN were involved in efficient phagocytosis of the bacteria as well as multimerization of DC-SIGN. Together with the demonstration that DC-SIGN mediated phagocytosis in these cells was sensitive

towards known downstream processors of phagocytosis, Syk kinase, Raf kinase, and NF-kB, it is convincing that DC-SIGN is a phagocytic receptor [238]. In addition, DC-SIGN activation triggers Rho-GTPases [239].

Dectin1:

Dectin-1 was identified to modulate T cell function [240] and subsequently found to bind β-glucans, what is expressed on primarily funghi, but also some plants and bacteria [241,242]. Similar to FcγRs Dectin-1 contains an ITAM-like motif, suggesting involvement of Src kinases. However, the Src kinase inhibitor PP2 did only partially inhibit internalization. Also different to FcγRs, a membrane proximal tyrosine residue embedded in the ITAM-like motif is involved [243]. Similar to FcγRs, Cdc42 and Rac1 are involved, but not Rho [243].

It was demonstrated that Syk was not involved for Dectin-1 mediated phagocytosis in macrophage [243]. However, Rogers *et al* demonstrated that Syk was partially involved in DCs. Thus Dectin-1 mediated phagocytosis differs between cell types. Whether this holds true for other proposed phagocytic receptors, such as MR, is not known. But it could explain the observations with the Mannose receptor in different cell types. The possibility that also additional molecules associate with Dectin-1 to initiate phagocytosis is possible, since immunoprecipitation experiment demonstrated that CD63 bind to Dectin-1 [244].

TLR:

Whether TLR4 or other TLRs are able to initiate phagocytosis is not known. However, TLR4 signaling induce both Rho GTP-binding proteins Cdc42 and Rac [245]. Thus it is still plausible that TLR4 signaling results in initiation of phagocytosis. This is currently still under debate.

Conclusion:

Bacterial-derived carbohydrate structures are the main constituent of the extracellular surface of bacteria. The recognition of carbohydrate structures induce uptake of the bacteria. This is important for antigen presentation, since efficient uptake delivers more antigens, increasing presentation of antigenic peptides towards the adaptive immune response. In other words, the efficiency of the innate immune response determines the efficiency of the specific adaptive immune response during infection. Bacterial internalization is a complex mechanism involving a variety of receptors and opsonins. The heterogeneity of phagocytic mechanisms allows the optimization of bacteria recognition, phagocytosis, and killing. The membrane-incorporated PRRs allow cell-specific innate immune responses, allowing different responses. As indicated by different pathways used in different cells by the same receptor (Dectin-1) [243,246]. In contrast, soluble PRRs, for example the collectins, are important for recognition of bacteria also outside tissue and facilitating uptake by different cell types.

Strikingly, experiments with BMDCs from MR-deficient mice demonstrated that MR-mediated endocytosis was essential for cross-presentation but dispensible for MHC II-restricted presentation of the model antigen OVA [98]. An additional study pinocytosis inhibition by dimethylamiloride (DMA) abrogated activation of OT-II cells in a dose-dependent and MR-independent manner, indicating that only pinocytosed and not MR-endocytosed OVA was used to activate the $CD4^+$ T cells. In contrast, the OT-I cells were unaffected by DMA [114]. This indicates that the constituve pinocytosis of small doses of OVA in DCs was specific for $CD4^+$ T cell activation, whereas MR^+ DCs could simultaneously internalize large amounts of OVA exclusively for cross-presentation. Furthermore, fluorescent microscopy experiments demonstrated that pinocytosis and MR supplied distinct intracellular compartments. MR-endocytozed OVA was directed into stable early endosome compartment that was excluded from late endosomes and lysosomes [114], known to be involved in cross-presentation pathway [115]. In contrast, pinocytosed OVA was transported exclusively toward lysosomes. Co-localization of pinocytozed OVA with lysosomal MHC II and MR-endocytozed OVA with MHC I, indicates that the subcellular antigen localization correlated with the selectivity of its presentation. Thus these findings suggest that differential antigen uptake by different receptors can already have major influences on subsequent T cell responses [114]. Indeed, targeting OVA to the CLR DEC-205 by conjugating OVA and a specific DEC-205 antibody result in cross-presentation of OVA,

whereas normal OVA lead to presentation via MHC class II molecules[247].
This is supported by another study that showed that differential expression of activating and inhibitory FcγRc during DC maturation critically influences the cross-presentation of antigen-antibody complexes [248]. FcγRI and FcγRIIa positively affected cross-presentation, whereas FcγRIIb act as a negative regulator of cross-presentation at the level of uptake[248]. The rapid and marked decline in FcγRI expression explains the overall reduction in FcγR-mediated phagocytic function associated with DC maturation [248]. To induce cross-presentation, the signaling motif ITAM is necessary. This ITAM-dependent pathway in its turn is critically dependent on DAP12, FcγR adaptors, and Vav guanine-exchange factors (GEF) that controls radical oxygen production and cross-presentation of particulate antigens by DCs [249]. Also polyreactive immunoglobulins target peptides to compartments that recruit Rab27a and are involved with cross-presentation [127].
In conclusion, these findings demonstrate that recognition of carbohydrate structures on bacteria result in specific engagement of phagocytic receptors resulting in efficient uptake into specific compartments favoring specific antigen presentation.
The receptor dependence of specific antigen presentation process may allow presentation by only specific subsets of cells or cells in a specific stage of activation that express suitable receptors. The constitutive activity of pinocytosis seen in all DC subsets may ensure that cross-presenting DCs can acquire antigen for induction of cognate $CD4^+$ T cell help, essential for effective $CD8^+$ T cell responses.

§4: Contribution of carbohydrate structures present on bacteria to intracellular antigen sorting.

The dogma was for many years that PRRs specifically recognize molecular motifs that are only associated with pathogens. However, further research especially on ligands for lectins have discovered also many self ligands. DC-SIGN recognize ICAM-2 [250], ICAM-3 [250], butyrophilin [251], human milk bile-salt stimulated lipase (BSSL) [252], Mac-1 [253], carcinoembriogenic antigen (CEA) [254], and CEA-related cell adhesion molecule (CEACAM1) [255]. In addition, MR is able to recognize ligands via both the cysteine-rich domain and the multiple CTLDs. The cysteine-rich domain is involved in binding to the hormones lutropin and thyrotropin, the proteoglycans chondroitin sulfate A and B, and sulfated Lewis-type antigen-containing glycoproteins [256], while the CTLD are responsible for the binding of thyroglobulin, lutropin, myeloperoxidase, and lysosomal hydrolases [257,258]. The overlap in endogenous and exogenous ligands of CLRs indicate that there must be another mechanism of antigen sorting occuring at subcellular level to prevent self-antigen presentation. This is also supported by the marked different immune responses against different ab-opsonized thus FcR-mediated internalization antigens, and the fact that DC-SIGN-mediated uptake could result in both $CD4^+$ and $CD8^+$ T cell responses [259].
Once inside the cell, internalized vesicles obtain a unique composition of their membranes and content determined primarily through the types of receptors engaged during internalization. Key to the discrimination between self endogenous antigens and foreign antigens is the family of TLRs[260]. Most TLRs are expressed at the cell surface, TLR3, 7, 8, and 9 reside within endosomes, and TLR2 and 4 are often enriched in endosomes. This leads to increased TLR recognition of pathogen-associated patterns from phagocytosed pathogens. This colocalization in phagocytes between activation of TLRs and phagosomes raises the hypothesis that ligation of TLRs may modulate phagocytosis.
As mentioned before, TLR4 is identified as a member of the receptor-complex of LPS, which is the main polysaccharide component of the Gram-negative bacteria surface. TLR4 and its coreceptor MD-2 are specific for LPS recognition [167,168]. Two other glycoproteins are involved in LPS recognition by TLR4/MD-2, respectively lipopoly-saccharide-binding protein (LBP) and CD14. These glycoproteins are not essential but greatly increase cell sensitivity to LPS [261]. LPS is recognized with high affinity by LBP and CD14, which shuttle it to MD-2 bound to TLR4.
To date the consensus is that TLR4 signaling is important by modulating indirectly the process of phagocytosis. At first, TLRs are able to upregulate the phagocytic capacity of phagocytic cells by MyD88-dependent signaling through IRAK-4 and p38 towards a

number of phagocytic gene expression programs [262-264], such as modulation of genes involved in uptake of bacteria such as Fc and scavenger receptors[262,265]. Notably, stimulation of DCs with LPS triggers activation of actin cytoskeletal rearrangements that results in an increased rate of uptake at early time points [266]. Thus TLR4 stimulation resulted in enhanced delivery of exogenous antigens to the antigen presentation pathways. Additionally, TLR4 stimulation result in IFNγ secretion, which is known to give rise to the immunoproteasome and consequently enhance quantity of antigens for presentation via MHC class I molecules (see chapter MHC I) [46].

An efficient antigen-specific $CD4^+$ T cell immune response is induced only when the TLR and antigen are present on the same antigenic structure and are internalized into the same endocytic compartment [260]. This indicates a compartimentalized induction of TLR signaling, that function as a kind of barcode to allow discrimination at subcellular levels [260]. It was demonstrated that the TLR-tag allowed discrimination between apoptotic cells and dangerous bacteria, thereby favoring TLR-tagged content above self-antigens from apoptotic cells to be presentated via MHC molecules [260].

TLR-mediated modulation of phagosome maturation.

One way of TLR signaling to favor cargo of TLR-tagged phagosomes occurs at the level of phagosome maturation. Over time, phagosomes acquire new components through sequential fusions with endosomes[267]. Maturing phagosomes ultimately fuse with lysosomes for terminal degradation of the cargo and killing of internalized microorganisms. Notably, phagosome maturation is accompanied by a progressive decrease in phagosomal pH, which drops from around pH 5.5 in nascent phagosomes to around pH 4.5 in lysosomes [84]. This process occurs within most cell types, including non–'professional antigen-presenting cells' such as fibroblasts, but not including immature DCs, which seem to actively maintain a more alkaline pH within their phagosomes [268]. Hydrolytic enzymes resident in late endosomes and lysosomes have low pH optima, which ensures that their activities are confined to a particular stage in the endocytic pathway[269]. Phagosome acidification seems to be tailored to the functions of the particular cell type in which the phagosome forms. Whereas the outcome of phagosome maturation in macrophages is the killing of internalized microorganisms and complete degradation and clearance of phagocytic cargo, maturation of phagosomes in DCs serves to prevent complete degradation of cargo antigens such that MHC molecules can present them. Accordingly, a progressive decrease in phagosomal pH occurs over time in macrophages, whereas no significant acidification seems to occur in phagosomes maturing in DCs[268,270]. Thus an optimized maturation of only pathogenic antigen-containing phagosomes is essential to a proper immune response. The phagosome maturation, thus also the final immune response, is optimized by TLR signaling recognizing bacterial carbohydrate structures as foreign. Upon phagocytosis of microbial pathogens, surface TLRs, including TLR4 and TLR2, are recruited to the phagosome and become activated by microbial cell wall components, such as the previous mentioned LPS or peptidoglycan. A block in maturation of *E.coli*-GFP containing phagosomes occurred in $TLR2x4^{-/-}$ or $MyD88^{-/-}$ macrophages, as observed by a lack of localization with late endosome and lysosomal markers [263]. Inhibiting the mitogen-associated protein kinase (MAPK) p38 resulted also in a block in maturation of TLR-stimulating cargo-containing phagosomes[263]. Thus phagosome maturation is regulated by signals from TLRs through the adaptor protein MyD88 and the mitogen-associated protein kinase (MAPK) p38. It is known that p38 can modulate the rate of endocytic traffic by regulating activity of guanyl-nucleotide dissociation inhibitor (GDI) on Rab proteins[271], thus probably the fusion of the phagosome with late endosomes or lysosomes required for phagosome maturation is regulated by TLRs. Remarkably, increased phagosome maturation was inhibited by knockdown of the autophagy pathway protein ATG5, suggesting that TLR-induced recruitment of classical autophagy pathway proteins to phagosomes promoted their fusion with late endosomes or lysosomes[272]. Functionally, all microbial pathogens including *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium*, engaged TLRs during internalisation and were delivered to

lysosomes at an inducible rate manifested by increased clearance and phagolysosomal fusion.

TLR-dependent modulation of antigen processing.

Another mechanism involved in antigen processing dependent on TLR signaling was recently identified. Burgdorf *et al* found that endotoxin-free OVA was less favored to activate OT-I T cells in comparison to endotoxin-coupled OVA and demonstrated that this was based on TLR4- and MyD88-dependent relocation of TAP to early endosomes, which is proposed to deliver cytosolic peptides that were degraded by the proteasome to stable early endosomes for antigen presentation via MHC class I molecules [115].

In addition, a recent study using a large scale quantitative proteomics approach to analyze the changes in protein abundance induced on phagosomes by IFNγ identified 167 IFNγ-modulated proteins on phagosomes. The IFNγ-modulated phagosomal proteins included proteins expected to alter phagosome maturation, enhance microbe degradation, trigger the macrophage immune response, and promote antigen loading on major histocompatibility complex (MHC) class I molecules. A dynamic analysis of IFNγ-sensitive proteins by Western blot indicated that newly formed phagosomes display a delayed proteolytic activity coupled to an increased recruitment of the MHC class I peptide-loading complex. These phagosomal conditions may favor antigen presentation by MHC class I molecules on IFNγ-activated macrophages [273].

TLR-mediated modulation of peptide loading.

Finally, TLR signaling is also involved in MHC loading with peptides.

Biochemical investigation of enriched phagosomes from DCs that phagocytosed two types of microspheres (magnetic or plain), of which one was TLR ligand-positive and the other TLR ligand-negative, demonstrated that the p31 isoform of MHC II-associated invariant chain was consistently degraded in HEL/LPS phagosomes, but persisted in HEL phagosomes despite the concomitant presence of LPS in magnetic phagosomes within those cells. The pattern of invariant chain degradation was also observed in phagosomes enriched from DCs that had phagocytosed HEL or HEL/LPS microspheres alone. These data suggest that p31 invariant chain degradation is confined predominantly to phagosomes that contain TLR ligands, thereby increasing peptide loading inside this confined compartment and thus favoring only antigens present in the TLR-tagged phagosomes. Accordingly, the levels of mature SDS-stable αβMHC II dimers were highly enriched in HEL/LPS phagosomes, in contrast to HEL phagosomes[260].

Another way to enhance peptide loading is to increase the amount of newly synthesized MHC molecules. LPS pretreatment is able to promote the IFNγ signaling pathway through triggering type I IFN production [274], which induce expression of MHC class II molecules via CIITA[2]. However, *de novo* MHC class II synthesis shuts down in LPS-treated DCs and macrophages as a result of transcriptional inactivation of the CIITA gene via histone deacetylation dependent on TLR4 or TLR2, ERK, and p38 MAPK [275,276]. Indicating a model in which MHC class II molecule synthesis is allowed in a defined period of time.

Important for antigen presentation is the control of MHC-peptide complexes that are present on the plasmamembrane, allowing activation of T cells. During LPS-induced maturation of the DC-cell line KG-1, these cells relocate MHC I-peptide complexes from the Golgi compartment to the plasma-membrane and these complexes were presented for a longer time at the cell surface [51]. Although the later is probably due to the decrease in endocytosis during DC maturation, thereby prolonging MHC I-peptide complexes presentation. The transient delay in trafficking of MHC I-peptide complexes towards the plasmamembrane may preclude internalization and degradation, explaining the increased steady-state levels of MHC class I observed early after initiation of maturation[51]. This seems similar for MHC class II molecules, because it was observed that DCs express more MHC II-peptide complexes after maturation [277]. This was attributed to different processes. At first, the previously described enhanced synthesis of MHC class II molecules and increased invariant chain processing and MHC II peptide loading, result in increased expression of MHC II-peptide complexes. Secondly, recruitment of MHC II molecules

from the MIIC to the cell surface upon T-cell engagement [33,277] or the retrograde transport via tubules of newly formed immunogenic MHC II–peptide complexes upon stimulation of APCs with microbial products or inflammatory mediators such as LPS [278]. Thirdly, extended half life of MHC II-peptide complexes by a decrease in endocytosis. As discussed in the introduction, recent studies demonstrated that immature DCs oligo-ubiquitinate conserved lysine residue within the cytoplasmic domain of MHC II-β after proteolytic processing of MHC II-associated invariant chain [35,279] in addition to MHC endocytosis that is mediated by a conserved dileucine-based signal in the COOH terminus of the cytoplasmic domain of MHC II-β chain[34,35]. Ubiquitination of mature MHC II is required both for efficient uptake from the plasma membrane and sorting at multi-vesicular bodies into luminal vesicles [35]. Together, these two sorting steps result in efficient intracellular retention of peptide-loaded MHC II, thereby preventing premature antigen presentation by immature DCs. Upon TLR4-dependent maturation, oligo-ubiquitination of the MHC II β-chain ceased, resulting in extended half life for MHC II-peptide complexes [35]. In parallel, empty MHC II molecules and MHC II-invariant chain molecules are constitutively endocytosed similar as in immature DCs[35].

Conclusion.

In conclusion, the context of an antigen (such as bacterial-derived polysaccharides) that interacts with TLRs is essential to optimize the process of antigen presentation by influencing selection, processing, and loading of antigens at a confined compartment. In addition, the interaction of antigens and TLRs is important to enhance the antigen presentation potential. This occurs not at a confined compartment but function at cellular level. This allows usage TLR ligands to enhance antigen presentation of several other antigens, e.g. as adjuvant. Indeed, the chemically detoxified derivative of native Lipid A from Salmonella minnesota R595 (MPL) is major constituent of GlaxoSmithKline's proprietary AS02 and AS04 adjuvants [280,281].

§5: Contribution of bacterial-derived carbohydrate structures recognition to T cell activation

Antigen-specific proliferation of helper T cells occurs only in response to antigen peptides in the context of the same MHC haplotype as the T cells. The TCR that is responsible for the antigen specificity of T cells is a heterodimer composed of either α and β, or γ and δ chains. These chains are organized into variable (V) and constant (C) domains, similar to BCRs with the V domain containing hypervariable regions. For downstream signaling, the T cell is associated with CD3. This accessory molecule is a complex of polypeptide chains that is required for TCR expression on cell surface and drives further downstream signaling after interaction of a T cell with presented antigens. In addition, it does not influence interaction with the antigen. The CD3 molecules contain an immunoreceptor tyrosine-based activation motif (ITAM) that can be found in multiple receptors and interacts with tyrosine kinases and play a major role in signal transduction of the immune system.

Although recognition of antigen-MHC complexes is mediated solely by the TCR-CD3 complex, signaling solely through the TCR results in a nonresponsive state (anergy) in which T cells are refractory to restimulation[282]. In order to prime T cells, accessory molecules are essential by strengthening or weakening the interaction between T cells and APCs. These molecules expressed at the APC surface are so-called co-stimulatory molecules. The requirement of inducible costimulatory molecule expression for T cell priming prevent T cell priming against self-antigens, because expression of costimulatory molecules is determined by relatively small number of stimuli, for example products of pathogens. Finally, priming of naive T cells to undergo clonal expansion and develop effector function requires a last signal. This third signal is variable; they can be membrane proteins as CD40-CD40L, or soluble factors such as cytokines excreted by them or neighboring cells. This third signal determines whether the naïve T cells become tolerant or fully activated T Cells and determines the differentiate state of the T cells. They make the greatest contribution when antigen levels are low. At high Ag levels extensive proliferation of T cells can occur in

the absence of a third signal, but T cells than don't become fully functional [283].

TLR-dependent costimulation.
Next to the above discussed control of TLRs on the first signal to T cells, e.g. antigen presentation in context of the MHC molecules, also the other signals involved in T cell activation are controlled by TLRs. Expression of costimulatory molecules on DCs is demonstrated to be triggered by TLR signaling pathways. Specific stimulation of TLR2 with LC1013 resulted in an higher expression of different costimulatory molecules, such as CD80, CD83, CD54, and CD40 [284]. As expected TLR2- or MyD88-deficient mice were unable to upregulate surface expression of several costimulatory molecules and MHC class II molecules upon stimulation with peptidoglycan [285]. Therefore, thus far I've showed that the engagement of TLR signaling pathways in DCs controls two key parameters necessary for optimal T-cell activation, the presentation of peptides within MHC class II and the expression of costimulatory molecules [286]. Collectively, these findings indicate that the discrimination of phagocytosed cargo at the subcellular based on the presence or absence of TLR ligands, result in a compartmentalised generation of peptide–MHC class II complexes where the contents of phagosomes derived from microbial pathogens are preferentially presented in the context of costimulation.

TLR-dependent cytokine expression.
Additionally, TLR signaling also induce cytokine expression by the Toll-interleukin 1 receptor domain-containing adaptor protein (TIRAP)-MyD88 pathway, or the Toll-receptor-associated molecule (TRAM)–Toll-receptor-associated activator of interferon (TRIF) pathway, respectively [287].
Many of these cytokines are also involved in determining differentiation fate of T cells upon activation, e.g. signal 3. Most TLR signaling was demonstrated to induce expression of the inflammatory cytokine IL12, IL18, and type I IFNs, skewing CD4 T-cell differentiation into Th1 cells [288]. Studies over many years showed that distinct PRRs can be engaged simultaneously or consecutively and they act synergistically or antagonistically in these different combinations, thereby leading to distinct responses as illustrated by distinct responses after TLR2 stimulation.

Recent studies have established the pivotal role of glycan-recognition by lectins contributing to the differentiation of T cells. In response to peptidoglycans, TLR2 in collaboration with dectin-1, or by itself, induces profound IL23 responses [289], while TLR-2 alone triggered by Pam3CysSerLys4 limits IL23 and promotes Th1 polarization [290]. In addition, binding of fungal β-glucans to Dectin-1 can directly or in synergy with TLR2 trigger secretion of cytokines, such as IL10, TNFα and IL12. This occurs in a pathway dependent on the functional ITAM-like motif in Dectin-1 that recruits tyrosine kinase Syk, that result in NFκB activation and subsequent cytokine response via Malt-1 and the Card9-Bcl10 complex. Thereby DC maturation is promoted and Th17 responses are induced [291].
Notably, it is also recently demonstrated that Dectin-1 stimulated DC were capable of converting mouse $CD25^{+}Foxp3^{+}$ regulatory T cells into $Foxp3^{+}ROR\text{-}\gamma t^{+}$ IL17-producing cells (Th17 cells) via IL23 secretion[292].
Moreover, although DC-SIGN lacks known signaling motifs, also DC-SIGN is identified as a signaling receptor. Triggering of DC-SIGN by ManLAM activated the serine and threonine kinase Raf-1, independently of TLR signaling. Activation of Raf-1 led to acetylation of p65, one of the key activating subunits of NFκB, but only after TLR signaling had activated NFκB. Strikingly, acetylation of p65 prolonged transcriptional activity of NFκB and enhanced the transcription rate from the IL10 gene. This pathway was found to be central to modulation of TLR-specific immune responses elicited by DCs in response to mycobacteria, fungi, and viruses [293].

Conclusion.
In conclusion, the interplay among innate signals and shaping of the adaptive responses is the consorted action of many players that influence each other. Whereas TLRs influence antigen processing and presentation regulated by CLRs, CLRs as antigen uptake receptors, on their turn may influence signaling pathways initiated by TLRs. The ultimate outcome of this concordant action is dictated by the signatures present on the pathogens (i.e. bacterial carbohydrate structures and TLR agonists) that determine which set of innate receptors are involved in shaping the immune repertoire of the DC.

Zwitterionic capsular polysaccharides: T-cell dependent antigens

§1: Immune response to capsular polysaccharides

As demonstrated in previous chapters, carbohydrate structures from bacteria play a pivotal role in the adaptive immune response against the bacteria, since recognition by innate receptors contribute to efficient antigen presentation and fine-tuning of the adaptive immune response.

An optimal immune response that is able to render a long lasting memory against bacterial polysaccharides is hampered by molecular mimicry: many infectious bacteria contain surface carbohydrates that are very similar in chemistry and/or structure to mammalian surface glycoproteins and glycolipids. As a result of this similarity, some pathogens evade immune surveillance because of a biochemical tolerance of those structures. An example of molecular mimicry is the a2→8-linked sialic-acid capsule of group B *Neisseria meningitidis* and the homologues mammalian neural cell adhesion molecule (N-CAM) [294]. Also the diversity of carbohydrate structures present on bacteria is enormous and hampers a specific and selective immune response. Bacteria render this diversity by slight genetic alterations or rearrangements in clusters of the genes involved in the synthesis of a particular polysaccharide. This results in a marked diversity in the monosaccharides composing the oligosaccharide repeating unit. Also other important variations contribute to a polysaccharide's biological specificity, including variations in the glycosidic linkages, the configuration of the anomeric center of each sugar, and the polymer's conformation [295]. Therefore, most polysaccharide antigens are unable to induce germinal center formation, memory responses, and affinity maturation antibodies.

In immunology, an antigen can be classified either as T-cell dependent or T cell independent (TI) antigen. T cell dependent antigens are antigens that require stimulation from helper T cells to elicit an immune response, as usually proteins and peptides are. The T cell dependent antigen is presented to the helper T cells via the MHC class II molecule with additional signaling molecules to ensure an long lasting immune response by generation of memory B and T cells. The antibodies against these T cell dependent antigens are of high affinity and multiple isotypes, IgA, IgM, IgG_1, IgG_{2a}, IgG_{2b}, respectively.

In contrast to the T cell dependent antigens, TI-antigens do not give rise to immunologic memory neither they do require T cells to induce an immune response. The TI-antigens are further divided into type 1 and 2 antigens based on their interaction with B cells [296]. TI type 1 antigens are capable of inducing proliferation and differentiation of naïve as well as mature B cells. A common example is bacterial LPS.

In contrast, TI type 2 antigens do not exhibit intrinsic B cell stimulating activity. In addition, these antigens are characterized by poor *in vivo* degradability and inability to stimulate MHC II-restricted T cell help [297]. TI type 2 antigen-specific antibody production is induced by activation of mature B cells via cross-linking of the antigen with the B cell receptor. However, it seems only to function in certain situation. A characteristic of the TI type 2 antigens is namely that they cannot stimulate the immune responses in children less than two years old and the elderly [298]. In the case of infants it is explained by the delay in capability of antibody formation. Humoral immunity in early life as expressed in the ability to synthesize immunoglobulin (Ig) shows a gradual development to adult Ig concentrations although the various Ig isotypes develop with a different rate. Antigens of protein nature can effectively induce an antibody response in the first 2 years of life. However, antibody synthesis towards polysaccharide antigens of encapsulated bacteria is absent or low. This cause a condition which makes infants and young children highly susceptible for infections with encapsulated bacteria, as indicated by *S. pneumoniae* and *N. meningitis*. Pneumonia and meningitidis caused by encapsulated bacteria show the highest incidence in the age of 4-18 months.

To overcome the problem of lack in memory induction, polysaccharide targets were coupled to carrier proteins possessing T cell peptide epitopes [299,300]. The idea is that the polysaccharide-protein conjugates bind to the

B cell receptors of polysaccharide-specific pre-B cells and are subsequently presented via classical MHC class II pathway to αβ $CD4^+$ T cells. In its turn, the activated helper T cell secrete cytokines to stimulate B cell maturation and induce immunoglobulin class switching [301]. The value of the glycoconjugate vaccines that are in clinical use has been decisively established [302]. However, there are addressed some considerations with the current-generation glycoconjugate vaccines. Current conjugation chemistry requires polysaccharide modifications that alter natural epitopes, with consequent generation of low-affinity antibodies against the native polysaccharide. In addition, the structural heterogeneity of glycoconjugate vaccines is concerning. The random conjugation hampers reproduction from batch to batch. Another concern is the phenomena of carrier-induced epitope suppression (CIES). Individuals show inhibited immune responses to the polysaccharide, when immunized with a glycoconjugate vaccine that contains a protein that was previously used for immunization of the individual. The major problem is still that glycoconjugate vaccines do not induce the most efficient immunity; most of them requires booster vaccines, individuals obtain a relatively short duration of immunity, and more important the glycoconjugate vaccines have a poor immunogenicity in the immunocompromised group of elderly and patients with underlying B cell defects.

Intriguingly, in recent years have paradigm-shifting observations been made in which contrary to prior concepts, zwitterionic polysaccharides (ZPSs) activate T cells in the absence of carrier proteins[303-305]. The coming paragraph will address the recent observations on ZPSs, implications, and future directions in ZPS-related research.

§2: Zwitterionic polysaccharides

The ZPS T-cell activating role was sequentially discovered by analysis of the role of anaerobe *Bacteroides Fragilis* in formation of abscesses. The formation of abscesses in the abdomen is one of the most common problems encountered after surgical procedures that involve the peritoneum. The most abundant microorganism isolated from such abscesses after surgery or physical trauma to the intestine is the Gram-negative anaerobe *B. fragilis* [306]. In the animal model for intra-abdominal abscess formation, the surgical implantation of pure cultures of *B. fragilis* and sterile caecal contents induced the formation of abscesses in laboratory rats[307]. Studies of the virulence factors responsible for abscess formation by this organism showed that the capsule of polysaccharides of *B. fragilis* potentiates the development of this host response in the rat model. Transfer of serum antibodies from rats immunized with this capsule from *B.fragilis* did not prevent the development of intra-abdominal abscesses, but only prevent *B. fragilis* infection [308]. However, splenocytes from immunized rats did prevent development of intra-abdominal abscesses [309]. Further investigation showed that the T-cells have the ability to prevent development of intra-abdominal abscesses [303]. The sterile caecal content did not induce abscesses, but abscesses were induced by $CD4^+$ T helper cells that prior to adoptive transfer to the peritoneal cavity of the rats are exposed to the zwitterionic polysaccharide of *B. fragilis* named Polysaccharide A (PS-A)[303,310]. This polysaccharide is the most immunodominant constituent of the capsular polysaccharides. In addition, it was soon shown that although PS-A and sterile caecal contents together induced pathology, administration of PS-A alone protected animals against it following challenge with *B. fragilis* and other abdominal-present bacteria [303]. Additional studies demonstrated that purified PS-A induced a dose-dependent proliferation of $CD4^+$ T cells when cultured in presence of APCs[311,312]. Characterization of the T cell response revealed that the $CD4^+$ T cell response was 2.4 times greater than the $CD8^+$ T cell response[311], indicating that specific T-cell populations are involved. The requirement for APCs in this system is again established by showing that depletion of MHC class II-bearing cells abrogated the proliferative activity of PS-A. The loss of activity following antibody blockade of MHC class II molecules suggested that available class II molecules are required for PSA-mediated T cell activation[311]. Studies with confocal microscopy showed colocalization of MHC class II molecules, together with PS-A and αβ-TCR on the surface of $CD4^+$ T cells suggest that PS-A induces APC-T cell engagement through binding to both molecules[313]. Further research identified that monocytes,

dendritic cells, and B cells are all able to serve as APCs for ZPS-mediated T cell activation[305]. Also other polysaccharides are identified that elicit a potent $CD4^+$ T cell response in vitro and confer protection against abscess formation, Sp1 of *S. pneumoniae* [314,315], CP from types 5 and 8 *S. aureus*[310], and PS-A(1&2) from *B. fragilis* [312,314,316]. The capsular polysaccharide Sp1 of *S. pneumoniae* is incorporated in a recently polysaccharide-based vaccine named PneumoVax (e.g. Prevnar or Pcv7).

§3: ZPS structure

T cell-activating polysaccharides vary significantly in their monosaccharide compositions, linkages, and sequences. The only obvious common feature is their zwitterionic charge motif. Each zwitterionic polysaccharide (ZPS) carries a high density of positively charged amino and negatively charged carboxyl or phosphonate groups. The dual charge motif is generally rare among naturally occurring polysaccharides[317].

Sp1 is composed of galacturonic acid and 2-acetamido-4-amino-2,4,6-trideoxygalactose, whereas PS A2 contains fucose, mannoheptose, 2-amino-4-acetamido-2,4,6-trideoxygalactose, 3-acetamido-3,6-dideoxyglucose, and 3-hydroxybutanoic acid[317]. CP from type 5 and 8 of *S. aureus* its repeating unit is composed of 2-acetamido-2-deoxy-L-fucose, 2-acetamido-2-deoxy-D-fucose, and 2-acetamido-2-deoxy-D-mannuronic acid[318], distinct by different linkages[319]. PS-A2 is zwitterionic and carries one cationic free amine and one anionic carboxylate in each repeating unit. It forms an extended right-handed helix with two repeating units per turn and a pitch of 20 Å. Positive and negative charges are exposed on the outer surface of the polymer in a regularly spaced pattern, which renders them easily accessible to other molecules. The helix is characterized by repeated large grooves whose lateral boundaries are occupied by the charges. The overall conformation of Sp1 is an extended right-handed helix with eight residues per turn and also a pitch of 20 Å. The molecular surface is covered with a high density of both positive and negative charges. Within each repeating unit, the two carboxyl groups are spaced 4.6 Å from each other and positioned on one side of the helix, whereas the amino group is positioned on the other side. The distances between the amine and two carboxyl groups in one repeating unit are approximately equal, whereas the carboxyl groups from different repeating units are further apart. The positive and negative charges are located approximately in analogous positions along the polymer chain but face opposite directions[317]. The glycosidic oxygen atoms of Sp1 can be superimposed onto the glycosidic oxygen atoms of PS-A2 with an RMSD of 1.6 Å. This reveals that these two ZPSs have essentially the same backbone conformation. In addition, the spatial arrangements of the amines in the two polysaccharides display a very similar zig-zag fashion with the amines pointing outwards. The average distance between adjacent amines in Sp1 is 14.6 Å, which is very similar to that of PS-A2 (15.5Å). Even when Sp1 and PS-A2 are superimposed solely on the basis of their amino groups, the overlap of their backbone structures is still evident [317]. Both helices of Sp1 and PS-A2 are characterized by repeated charged grooves. Roughly one helical turn defines one groove on one side. The grooves are approximately 14 Å wide, 10 Å long, and 5 Å deep. The size of the grooves of Sp1 is comparable to that of PS-A2[317,319]. The positive charges are located along the lateral groove wall, while the next positive charged residue is part of another groove located on the other side of the helix. The negative charges occupy the floor and two sides of the groove and are shared by adjacent grooves[317]. The similarity in overall structure and presentation of charges and lack of similarity in composition, indicates that the zwitterionic charges are likely to be involved in T-cell activation by ZPSs. This is strengthened by the findings of several reports discovering varies zwitterionic glycosphingolipids that exhibit immunomodulatory biologic activities on peripheral blood mononuclear cells, as well as on T- and B-lymphocyte populations[320-323].

Indeed, PS-A and not N-acetylated PS-A elicit a dose-dependent activation of human T cells in vitro, indicating that free amino groups that are removed by N-acetylation on PS-A are critical for T cell activation[303]. In addition, conversion of this group to a tertiary amine that still retains its positive charge by treatment with an aldehyde group under reducing conditions, significantly reduced in vitro T cell proliferation. Also chemically modification of

the carboxyl group associated with the pyruvate substituent on PS-A via carbodiimide-mediated reduction, resulted in a 72% decrease in the proliferative response of T cells as compared with the unmodified PS-A[303]. Similar, conversion of the free amino group of Sp1 to a tertiary dimethylamine, that retained its positive charge, resulted in a significant decrease in the proliferative activity[303]. The general conformation indicates two models for interaction with proteins. One scenario could be that the ZPS binds to other molecules primarily ‘‘along its sides’’ where they display a high density of alternating opposite charges. High binding affinities would be achieved via abundant electrostatic interactions supplemented by the potential for numerous hydrogen bonds to hydrophilic hydroxyls and, to a lesser extent, van der Waals interactions. In the second scenario, the grooves of PS-A2 serve as the primary binding domains. The geometry of each groove would be able to accommodate the insertion of an α-helix from a protein. The “groove-binding model” is supported by an *in silico* analysis that demonstrated that hypothetical α helices (10–14 mers) with charged side chains at their termini fit very well into PS-A2 grooves. The charges at the edges of a groove would help anchor the polysaccharide. In addition to multiple salt bridges, the complex would be stabilized by primarily hydrophobic interactions along the inner surface of the groove [324].

In either of these scenarios, charged groups are located at critical positions and contribute significantly to binding, which would explain why charges are essential determinants of the biological activity of ZPSs.

Since crystallography studies have demonstrated that α helices form the lateral boundaries of antigen binding clefts in MHC molecules, the ‘‘groove-binding model’’ is the favored model for T cell stimulating activity of ZPSs.

Whether positive and negative charges contribute equally to the T cell activation function of ZPSs and whether repeating units are required for this function are still unresolved questions. The chemical treatments of PS-A demonstrate that both positive and negative charges are necessary [303]. For the later question, the correlation between size of the ZPSs and its binding to MHC class II molecules was determined [325]. Native PS-A with a high molecular weight bind only modestly to MHCII molecules. However, after minimal 15 minutes of ozone treatment, the fragments were able to bind quite well. The binding of PS-A to MHC class II molecules peaked at 45 minutes of ozone treatment, which correlates to a fragment size of about 3 to 10 kDa or three or ten repeating units. This agrees with an earlier study that demonstrated ZPSs smaller than 5 kDa fail to activate T cells [326]. However, once the fragment size of PSA is reduced to approximately 1 kDa (one repeating unit), the binding functionality was lost [325]. Since the conformation of a native and 3kDa fragment of PS-A showed similar helicity, as observed by circular dichroism measurements, it is thought that it is not a lack of helicity but a combination of potentially masking the binding domain in a higher order structure that is not readily apparent by CD measurements and unfavorable thermodynamics [325].

Additionally, neutralization of the positively charged free amines resulted into a striking conformational change that was similar to random coils and was accompanied with near ablation of the binding function of PS-A 3kDa fragment to MHC class II molecules[325]. Controversially, neutralization of the negatively charged carboxylate group resulted in a conformational change and abrogation of binding, but the structural change appeared to be simply a reduction in helical content. In addition, since the positive charges of the PS-A helix are pointing outwards, N-acetylation would not be expected to result in a conformational change. However, N-acetylated PS-A was shown to have lost the binding activity to MHC class II molecules [303]. Collectively, these data indicate that the zwitterionic charge motif is necessary for the maintenance of a helical conformation of PS-A. This implies that PS-A structure and function are closely correlated and PS-A must maintain significant helical content as well as the zwitterionic charge motif in order to enable binding to MHC class II molecules.

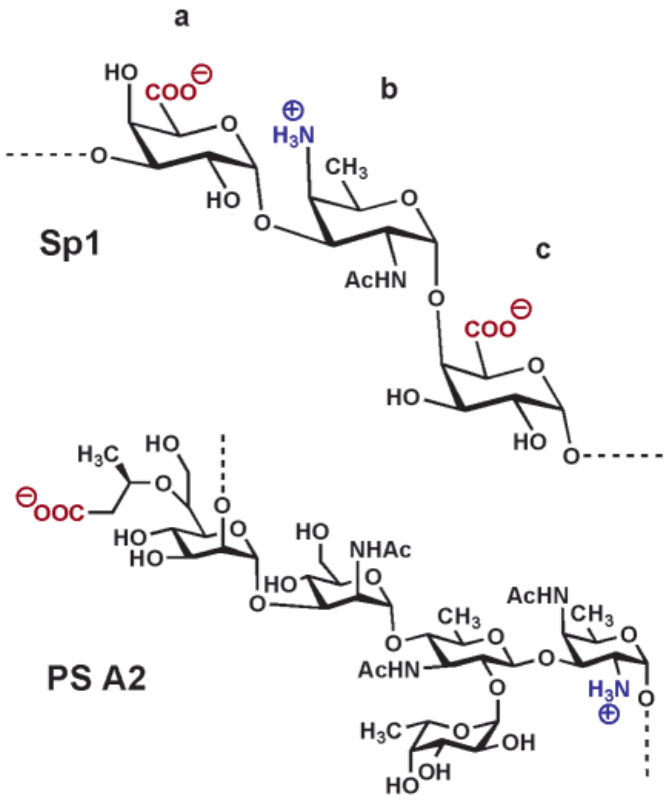


Fig.2. Schematical depiction of the molecular structure of Sp1 and PS-A.

§4: ZPS processing

T cells were the major players in development of intra-abdominal abscesses, therefore it was not surprising that the PS-A induced development of intra-abdominal abscesses via T cells. Early studies showed that MHC class II molecules were essential for T cell activation, similar to activation via peptide antigens. At first, it was shown that APCs were necessary to activate T cells [303]. In the same report it was also demonstrated that ZPS-mediated T cell activation requires the involvement of the αβT cell receptor (TCR) since the addition of

antibodies specific for this ligand inhibits T cell activation by ZPS [303]. Another article demonstrated with a transwell experiment that contact between DC and T cell is necessary for T cell proliferation [312]. With blocking antibodies it was demonstrated that available MHC class II molecules were required for T cell activation by ZPSs [312]. In addition, T cell activation by PS-A was abrogated by culturing with APCs lacking MHC class II molecules [312]. T cell proliferation assays with mononuclear cells as APCs and $CD3^+$ T cells as responders in the presence of blocking Abs to the MHC class I molecules HLA-A, HLA-B, and HLA-C, the MHC class II molecules HLA-DR, HLA-DP, and HLA-DQ, and their isotype controls demonstrated that only the MHC class II molecule HLA-DR mediated ZPS-induced T cell proliferation [312]. For Sp1 it was demonstrated to be a physical interaction between the ZPS and HLA-DR, since Sp1 co-immunoprecipitated with HLA-DR [312]. APCs of different HLA-DR haplotypes were able to stimulate proliferation of unrelated donor T cells, suggesting that ZPS may offer several different binding sites and consequently promote promiscuous binding to HLA-DR [312]. Additionally, fluorescent staining shows that Sp1 induced a twofold increase of HLA-DR surface expression on monocytes, which peaked at 8 h of incubation [305]. Induced HLA-DR expression was eliminated at 24 h of incubation. Incubation of B cells with Sp1 showed increased HLA-DR surface expression with similar kinetics, whereas immature monocyte-derived DCs showed HLA-DR surface expression upregulation after 48 h of incubation with Sp1. The increased surface expression of HLA-DR on ZPS-treated APCs correlated with specific mRNA transcription [305]. Decreased T-cell proliferation was

associated with decreased HLA-DR translocation to the cell surface. Collectively these findings indicate Sp1 and PS-A are presented on newly synthesized HLA-DR molecules. The interaction shows binding stoichiometry of one to one and allelic selectivity between DR1, DR2, and DR4 as measured by dissociation constants (Kd), thus suggesting that specificity and restriction could play an important role in carbohydrate-driven T cell responses[324]. Pre-processed PS-A fragments of approximately 15kDa bind sixfold and threefold higher to HLA-DR2, respectively than HLA-DR1 and HLA-DR4 [324].

However, the mode of interaction between ZPSs and HLA-DR were not established. ZPSs could interact with HLA-DR similar to regular peptide antigens as described in previous chapters, but it is also possible that ZPSs function as superantigens. Superantigens are MHCII-dependent molecules that are presented without processing as an intact protein bound out-side of the normal peptide binding domain, usually to MHCII molecules already loaded with a peptide [327]. In a competition assay, increasing concentrations of PS-A were added to MHC class II molecules that were preloaded with either peptide (MBPp) or superantigen (SEA). It was demonstrated that PS-A outcompeted peptide and superantigen by respectively 75% and 80%. This indicates that PS-A bind in the groove of the MHC class II molecules (similar to peptides) and outside the groove (similar to superantigens), or PS-A induces a conformational change upon binding to MHC class II molecule that inhibit binding of both peptides and superantigens. Further studies demonstrated binding of PS-A to MHC class II molecules was size-dependent, especially native PS-A was not binding that well, indicating that processing into smaller fragments is favoring MHC II presentation [303,326]. Kinetics studies investigating the influence of the duration of APC treatment with ZPS show that incubation times comparable to those for conventional antigen are required for the induction of T-cell activation, thus supporting the implication that time for intracellular processing and transport of the MHC-II and ZPS complex to the cell surface might be a prerequisite for T-cell activation [305]. Kinetic studies on in vitro proliferation experiments with T cells showed that both PS-A from *B. fragilis* and Sp1 from *S. pneumoniae* elicited a response that peaked on day 6, which lies between the fast proliferation kinetics of a superantigen (SEA) or a polyclonal mitogen (Con A), which peaks on day 3, and that of a nominal protein Ag (tetanus toxoid), which peaks on day 9 or 10 [315]. Concomitant *in vivo* analysis in which rats were injected s.c. with PS-A, KD20, or PBS as a negative control indicated strong correlation between in vitro and *in vivo* effects of ZPSs[315]. Analysis of Sp1-stimulated T cells demonstrated an increase of approximately half of the TCR Vβ chains, with an overall pattern that is distinct from the superantigen SEA, but similar to the polyclonal mitogen Con A. This suggests that ZPSs trigger polyclonal T cell responses[315]. These findings also demonstrate that ZPS binding to HLA-DR is not similar to superantigen, indicating that the conventional MHC II pathway is possibly used by ZPSs. This is supported by live cell imaging on immature DCs showing that a part of Sp1 internalize in compartment with Rab5 and BCECF-dextran, indicative for the early endosome. This is followed by maturation of the endocytic vesicle as demonstrated with subsequent colocalization with Rab7, dextran, lysotracker, and OVA[328]. There was no localization with Rab11b, indicative for recycling vesicles. Binding to MHC class II molecules is necessary for translocation of PS-A to the surface of APCs, since PS-A remains in intracellular compartments and cannot traffic to the cell surface in MHC II-deficient cells[329]. PS-A was also found to colocalize with MHC class II molecules in the MIIC compartment and LAMP-1[328,329].The pathway was sensitive to cytochalasin D and colchicines, thus PS-A was internalized actively[329]. Similar to the classical MHC II antigen presentation pathway, as described before in this manuscript, tubules were extending from the perinuclear area in which was intensely labeled for MHC class II molecules and Sp1[328]. Similar to the classical peptide antigen presentation pathway, further investigation demonstrate that Sp1 was exchanged for CLIP to get into MHC class II molecules and HLA-DM was required to catalyze this exchange [328]. Perhaps that HLA-DM is similar to peptide antigen presentation pathway involved for selection of proper length carbohydrates. It is known that this binding is sensitive to ionic strength and

pH, because both electrostatic bonds and ionizable groups are important in PS-A presentation. Comparing in vitro MHCII binding experiments of peptides and PS-A, PS-A binding was remarkably more sensitive to pH (i.e., preferring acidic pH) despite the fact that no significant change in net carbohydrate charge would be expected within that range. This indicates that cell entry is necessary for ZPS molecules to achieve presentation because the association requires an acidic environment [324]. Collectively, these findings demonstrate that ZPSs are internalized and enter the same pathway as peptide antigens. Both N-acetylated PS-A (negative charged) and carbodiimide-treated PS-A (positive charged) are internalized similar to zwitterionic PS-A. However, none of these PSs that are lacking the zwitterionic motif are presented on the surface of APCs [324]. Since the circular dichroism experiments demonstrated that helical content was determined by zwitterionic motif, it is unclear whether the zwitterionic motif is involved in binding or maintaining proper 3D conformation [325].

Peptide presentation requires processing into peptides by proteases. The optimal binding of smaller fragments in vitro to MHC class II molecules indicate that processing is also required. In the endosome compartment, some glucosidases are present but only at late compartments. In vitro experiments showed that neither low pH nor reducing conditions had a measurable effect of PS-A. However, oxidation by H_2O_2 at specifically pH 7.3 and not pH 5.0 reduced the size of PS-A[329], whilst in vitro binding to MHC class II molecules by PS-A did occur at lower pH and was reduced at pH 7.3[329]. This indicates that processing would occur in early endosomes prior to binding in later stage of endocytosis, likely by oxidation. Indeed, iNOS deficient splenocytes were unable to present native PS-A, but did present low molecular weight pre-processed fragments of PS-A to T cells, whilst NADPH oxidase (NOX2) deficient mice were efficiently presenting both native and pre-processed PS-A[329]. These findings indicate that APCs require iNOS for processing of PS-A and for activation of T cells. After exposure of APCs to cytokines or microbial products, iNOS is up-regulated and generates large quantities of nitric oxide (NO) by catalyzing the oxidation of L-arginine. NO is a short-lived radical that forms various NO-derived RNSs. NOX2 complex is a multimeric complex that generates superoxide by transferring electrons from NADPH inside the cell across the vesicular membrane and coupling these to molecular oxygen to produce the superoxide, which is a reactive free-radical[330]. iNOS is upregulated upon challenge with PS-A and correlate with increased NO production.

Further investigation demonstrate that N-acetylated PS-A, thus in which free amino groups have been blocked by N-acetylation, is degraded similar in both iNOS wt and iNOS-deficient mice, whereas native PS-A was degraded to a greater extent in wt DCs than iNOS-deficient DCs. This suggests that depolymerization of PS-A occurs via NO-dependent mechanism that requires free amino groups. Since it is known that deaminative depolymerization requires free amino or N-sulfo groups, but not the N-acetyl groups, these data indicates that the NO-mediated depolymerization is a deamination reaction [331]. Also in vitro T cell activation was dependent on this deaminative depolymerization of PS-A, since iNOS-deficient DCs did not induce T cell proliferation [331]. In contrast, incubation of iNOS-deficient DCs with pre-processed PS-A resulted in T cell proliferation [331]. Since it was previously demonstrated that the zwitterionic motif is required for T cell activation, it is conclusive that NO-mediated depolymerization does not alter the charges.

This NO-dependent process is supported by experiments with MPO-deficient monocytes or macrophages, in which large amounts of nitric oxide are generated and PS-A is more degraded[331]. Also in vitro experiments showed that deamination is important for ZPSs processing. NO-saturated solution significantly degrades PS-A at neutral pH, whereas N-acetyl PSA, which contains no free amino groups, is completely resistant to NO attack[331]. In contrast, peroxynitrite or its generator (SIN-1) did not have an effect on PS-A size. Thus RNSs, including NO and nitroxyl, are responsible in vitro degradation of PS-A through deamination. The consequent generation of approximately 10kDa predominant product after PS-A degradation mediated by primarily RNSs indicates that this process is finely controlled. Since it was demonstrated that the zwitterionic motif is a prerequisite for ZPS-mediated T cell activation, NO-mediated deamination of PS-A

does not disrupt the zwitterionic nature of PS-A [331].
To date, only a few ZPS specific T cell hybridoma's were generated, Sp1[332], and PS-A[315], respectively. The polyclonal T cells prevented against other ZPSs and not to non-ZPSs, thus indicating a cross-reactivity between ZPSs. *In vivo* protection was gained against intra-abdominal abscess formation upon challenge with *B. fragilis* [332]. This confirms the modulatory role of ZPS-specific T cells in formation of intra-abdominal abscesses. Moreover, chemical introduction of zwitterionic motifs in anionic polysaccharides generated zwitterionic polysaccharides that function quite similar in vivo as the natural ZPSs [333].

§5: Immunomodulatory role of ZPSs in intra-abdominal abscesses

Initial studies demonstrated that surgical implantation of PS-A together with sterile caecal content induce T cell activation that is essential for abscess formation[303]. Since studies have shown that engagement of different costimulatory pathways leads to markedly different T-cell responses, it is important to ascertain whether a requirement for costimulation exists and determine the type(s) of costimulatory pathways that are involved in Zps-mediated T-cell activation.
The interaction of CD28 on T cells with its ligands B7-1 and B7-2 on APCs is a major T-cell costimulatory pathway that controls the T cell response to a variety of antigens[334]**.** Ligation of CD28 with B7 promotes cell cycle progression and upregulates IL-2 mRNA at the level of both transcription and translation resulting in increased IL-2 expression[334]. Treating animals with CTLA4Ig, a fusion protein that effectively block CD28-B7 interactions[335], prior to challenge with PS-A, Sp1, and CP5, resulted in significant reduction of abscesses[303,311]. Demonstrating that T cell costimulation via CD28-B7 is necessary for ZPS-mediated T cell activation. Additionally, B7-2-specific antibodies prevented abscess formation while the B7-1-specific fusion protein did not, showing that the development of this host response is completely dependent on CD28–B7-2 interactions[303,305]. In addition, experiments using CD40 blocking antibodies demonstrate that upregulation of CD40 surface expression is not a prerequisite for ZPS-induced T-cell proliferation. However, binding of CD40-CD40L is required to sustain the bidirectional signals necessary for the initiation of T-cell proliferation mediated by ZPSs[305]. These findings show that ZPSs promote activation and proliferation of $CD4^+$ T cells via the first signal, TCR-MHCII-ZPS antigen engagement, which depends on retrogradely transported HLA-DR, and the second signal, that is provided by the costimulatory molecules B7-2 and CD40 on activated APCs. Indicating that ZPSs have potential to induce complete immune response. Treatment with Sp1 increased the expression of B7-1 and B7-2 on monocyte-derived DCs, on monocytes, and on B cells. After a 24-h incubation with ZPS, B7-2 surface expression was eliminated, while B7-1 expression was still increased at a low level. CD40 surface expression was induced neither on B cells nor on monocytes when incubated with Sp1 alone or with Sp1 in the presence of T cells[336].
The T cell activation resulted in profound T cell proliferation and associated IL-2 secretion, which are initial signals leading to inflammatory reaction, which culminates in abscess formation upon challenge with *B. fragilis* in naïve rats[315].
The mechanism that underlies abscess formation includes PS-A that was shown to stimulate production of chemokines, such as IL-8, from T cells and peritoneal macrophages [337]. This result in recruitment of polymorphonuclear leukocytes (PMNs) to the peritoneal cavity. Another mechanism is induction of pro-inflammatory cytokines such as TNF-α and IL-1α from peritoneal macrophages, which lead to cell adhesion molecule ICAM-1 to be expressed by mesothelial cells [337]. This result in tethering of IL-8-attracted PMNs into the inflamed tissue, which leads to further PMN recruitment and sequestration in the peritoneal cavity[304]. A recent study demonstrate that IL-6 is expressed upon stimulation with Sp1 by peritoneal macrophages and DCs in vitro[338]. IL-6 is a pleiotropic inflammatory cytokine that is produced by a variety of cells and acts on a wide range of tissues[339]. It stimulates the activation, migration, survival, and proliferation of $CD4^+$ T cells and acts on T cells as an anti-apoptotic factor[339]. Additionally, it has been linked to T cell-dependent immune diseases and autoimmune diseases such as inflammatory bowel

disease[340]. Upon stimulation with Sp1, IL-6-dependent migration of $CD4^+$ T cells into the peritoneal cavity induce intra-abdominal abscesses[338]. Sp1-induced IL-6 acts as anti-apoptotic agent on $CD4^+$ T cells and induce IL-17 expression in $CD4^+$ T cells[338]. The induction of IL-6 depends on the zwitterionic motif, since chemically treated Sp1 lacking this motif does not induce IL-6 expression[338].
The CD40 ligand interaction on T-cells with the CD40 on APCs is critical to elevate expression of other co-stimulatory molecules and the secretion of a variety of cytokines including IL-6, thus it is likely that the previously mentioned requirement of costimulatory interactions via B7–CD28 and CD40–CD40L for ZPS-mediated $CD4^+$ T cell activation and subsequent abscess formation contribute significantly to the up-regulation of IL-6 synthesis.
Recently, it was shown that $CD4^+$ T cells produce a limited amount of IL17 upon ZPS stimulation [332,338]. The primary source for IL-17 is a small specific subtype of $CD4^+$ T cells, namely Th17 cells. Th17 cells are known to be pivotal in immune responses against some funghi and bacteria. The Th0-cells differentiate into Th17 cells upon stimulation with IL-6 and TGF-β. So far, TGF-β is not investigated in formation of abscesses, but if it does it could indicate that Th17 cells are key players in the formation of abscesses. This indicates that next to pre-existing Th1 cells also Th17 cells are involved in abscess formation. It is possible that the initial Th1-mediated inflammation is attenuated by IFNγ, which modulate APCs and evolves toward a Th17-mediated chronic inflammation causing abscess formation. However, it is currently unclear which physiological conditions allow differentiation into Th17 cells next to pre-existing Th1 cells.

Subcutaneous application of different ZPSs prior to i.p. challenge with *B. fragilis* prevented subsequent abscess formation[309]. Additionally, it was found that treatment of ZPSs prior to i.p. challenges prevented abscess formation by various intra-abdominal inducing agents[311]. This cross-reactivity and general suppression of the host response that leads to abscess formation is also demonstrated by adoptively transferring ZPS-specific T-cell hybridoma's prior to challenge with *B. fragilis* in peritoneal cavity of the rat[315]. In addition to natural occurring ZPSs, also polysaccharides chemically modified to possess a zwitterionic motif are able to prevent abscess formation[309,311]. Also to this modulatory role of ZPSs in abscess formation play T cells a central role. Depletion of $CD4^+$ T cells from a preparation of splenocytes resulted in a loss of PSA-induced proliferation[311]. $CD4^+$ T cells isolated from animals that had been immunized with PSA had upregulated expression of the T-cell-activating cytokine IL-2[315]. Further investigation demonstrated that after s.c. Zps administration the protective function of T cells resulted from anergy, as indicated by the possibility to reverse the effect upon addition of IL-2[315]. Indeed, specific subtypes of $CD4^+$ T cells that are $CD45Rb^{low}$ were found to produce IL-10 upon ZPS engagement. IL-10 is an anti-inflammatory cytokine capable of inhibiting various inflammatory cytokines including IL-2. IL-10 was found in abscesses in the intra-abdominal rat model[341]. This finding was supported by another article reporting that Zps-stimulated T cells comprises an initial burst of IL-2, followed by the anti-inflammatory release of IFN-γ and IL-10[341]. The heterologous $CD45Rb^{low}$ $CD4^+$ T cell population includes activated T cells, $CD44^{high}$ $CD62L^{low}CD4^+CD25^+$ memory T cells, and naturally occurring $CD4^+CD25^+$ regulatory T cells[332]. The production of IL-10 and subsequent protection against abscess formation requires ICOS-ICOSL costimulation. Likely also the previous mentioned costimulation with CD40 or CD28 is involved, but this was not examined[336]. ZPS-treatment induced clonal expansion of the $CD4^+CD45Rb^{low}$ population [336].
In short, these findings show that certain bacterial capsules have the ability to modulate host immune responses in a T cell-dependent manner and offer an approach for preventing or treating deleterious Th1-mediated inflammatory host tissue responses in such as adhesions and inflammatory bowel disease in humans. Indeed, IL-10 that is produced by the $CD4^+CD45Rb^{low}$ T cells is responsible for ZPS-mediated protection against fibrosis [336]. In addition, the wildtype *B. fragilis* protects mice from experimental colitis induced by *Helicobacter hepaticus*, whereas a mutant strain of *B. fragilis* that lacks PS-A on its capsule, is not protective [342]. It was

demonstrated that germ-free mice had systemic T cell deficiencies, resulting in an imbalance towards the Th2 subtype. However, association of these mice with PS-A bearing *B. fragilis* corrected these T cell deficiencies, redressing the Th1/Th2 balance[343]. Simultaneously, the PS-A confers protection against overactive Th1 immune responses that result in IBD through production of IL-10 by $CD4^+CD45Rb^{low}$ T cells[342].

A recent article demonstrates that another mechanism confers protection against intra-abdominal abscess formation[344]. Characterization of the T cell influx identified a very small population of $CD8^+CD28^-$ T cells that was increased in both the spleen and in the peritoneal cavity upon stimulation with Sp1. Depletion of these $CD8^+CD28^-$ T cells resulted in an enlargement of the abscesses, indicating an important suppressive function in abscess formation. These T cells were expressing both TGF-β and IL-10, suggesting a mechanism dependent on these anti-inflammatory cytokines[344]. Conclusively, these findings indicate a mechanism in which $CD4^+$ T cell-mediated intra-abdominal abscess formation is controlled by regulatory subset of $CD8^+$ T cells, not excluding other relevant $CD4^+$ T cell mediated inflammatory responses. The induction of this cell population is independent of APC/T cell contact and APC-derived cytokines. However, TCR signaling was induced, as illustrated by enhanced Zap70 phosphorylation and NFκB translocation. Sp1 induced TCR signaling by enhancing crosslinking of the TCR, resulting in inhibition of apoptosis causing increased expansion of this cell population[344]. However, this model does not explain why other cell populations with similar TCRs are not expanding upon Sp1 stimulation. Whether different recognition of TCR Vβ-chain by Zps leads to various signaling events, as is the case for glycolipids and CD1 molecules , has to be established.

§6: Contribution of zwitterionic polysaccharides to antigen sorting

Contrary to PS-A that cause macrophages and DCs to secrete IL-12 and TNF-α, upon Sp1 these APCs does not secrete either of them[345]. This indicates a discrimination between both, whilst both structures and zwitterionic motif is similar. Investigation of IL-12 and TNF-α induction by PS-A stimulation, identified a requirement for TLR2 in macrophages and DCs[345]. This is not unexpectedly, since many of the immunologic events leading to PS-A-mediated T cell activation such as iNOS expression, MHCII presentation, costimulation, and cytokine production have been shown in other systems to be linked to TLR activation, as described before[346]. TLR2-deficient BMDCs internalize PS-A as efficiently as in wt BMDCs, indicating TLR2 is not involved in PS-A uptake. However, due to TLR2-deficiency up-regulation of MHC class II molecules, B7-2, and CCR7, and NO production, does not occur upon Sp1 stimulation[345]. As discussed before, these TLR2-mediated events are important in maximizing the adaptive immune response to PS-A by enhancing antigen presentation and subsequent T cell activation. The lack of TLR2 signaling results also in significantly reduced TNF-α, IFN-γ, which were essential to abscess formation. In addition, it is also demonstrated that PS-A induces IL12 production in DCs and that the induction of IL12 is substantially attenuated in TLR2−/− DCs. PS-A-induced IL12 production by DCs is vital for optimal IFN-γ production by T cells. This optimization of IFN-γ production facilitates the Th1 cell developmental process[280].

In conclusion, bacterial-derived zwitterionic polysaccharides now appear to be T-cell dependent antigens in contrast to the classical dogma so far in which bacterial polysaccharides were seen as T-cell independent antigens. The zwitterionic motif is essential for its modulatory role in the immune system. ZPSs function in both innate and adaptive arms of the immune system. In the innate immune system, stimulation through pathogen-recognition receptors (TLR2) of APCs result in NF-κB-dependent NO production. This is necessary for processing and presentation by MHC class II molecules, thus for initiation of the adaptive immune response. Additionally, ZPS stimulation up-regulates MHC class II molecules and costimulatory molecules on the APCs, enhancing the link between innate and adaptive immune response against these ZPSs. Upon antigen presentation in MHC class II molecules to αβ T cell receptors and costimulation, $CD4^+$ T cells become activated. This leads to a pro-inflammatory environment that recruits PMNs and provides help to B

cells, generating isotype switching and memory induction. Leading to protection of the host to the source of the ZPSs. To prevent chronic inflammation, the Th1-based inflammation is counteracted by IL-10 produced by ZPS-activated $CD4^{+}CD45Rb^{low}$ T cells and $CD8^{+}CD28^{-}$ T cells.

Discussion

In order to prevent autoimmune and/or an over-reactive immune response, fine tuning of the adaptive immune response is essential. The adaptive immune response comprises the humoral and cellular immunity. The main part of the humoral immunity is the antibody response, whereas a predominant part of the cellular immunity comprises the T cells. Collectively, the findings in this article indicate a general model in which innate signals are fine tuning the T cell responses via various mechanism, innate signals regulate each other, and in which T cells regulate T cell function in the adaptive immunity. To specify on the innate signals, as described previously there are three steps to a fully activated and specific T cell response. The first step includes the presentation of an antigen in the context of an MHC molecule to the T cell receptor. Antigen presentation is a tightly controlled process and the outcome determines whether it will be $CD4^+$ or $CD8^+$ T cells that become activated. The dogma for many years was that exogenous antigens were automatically presented via MHC class II molecules and cytoplasmic antigens via MHC class I molecules. However, recent studies identified cross-presentation that presents exogenous antigens via MHC class I molecules. Multiple pathways seem to exist in parallel, as depicted in figure 1. Further investigation could perhaps elucidate whether these different pathways co-exist at a same timepoint or state. It would also be important to investigate whether the distinct compartments in which cross-presentation is proposed to occur lead to distinct immune responses.

The classical view until the discovery of zwitterionic polysaccharides was that all capsular polysaccharides were T cell independent antigens. Since carbohydrates are the major constituent of the bacterial surface, presentation of bacterial antigens occurs often in presence of bacterial carbohydrates, or as recently discovered are the zwitterionic polysaccharides the antigen themselves. The bacterial sugar moieties are important by inducing actin reorganisation upon recognition by specific receptors at the cell surface that lead to efficient uptake of the antigen and delivery to compartments that intersect with pathways for MHC II presentation and cross-presentation. The MR-mediated endocytosis of OVA targets OVA to stable early endosome compartment, whilst without MR controlling OVA is delivery to lysosomal pathway. This clearly illustrates that distinct pathways of uptake results in distinct intracellular routing that favour either cross-presentation or presentation via the classical MHC II pathway. Furthermore, bacterial-derived carbohydrate structures influence processing of the antigen for presentation to T cells. As discussed, key to this are the Toll-like receptors. Blander and Medzhitov demonstrated that the processing is modulated only if the TLR-ligand and antigen are present on the same antigenic structure and are internalized in the same endocytic compartment. Together with the fact that TLRs only recognize highly specific components from pathogens, this shows that TLRs are able to modulate processing of selective antigens in a confined compartment. Indeed, TLRs exert positive and phagosome autonomous control on both the kinetics and outcomes of phagosome maturation. In addition, TLR signaling was shown to increase peptide loading on MHC class II molecules. This is reflected in the superior ability of phagosomes carrying TLR ligands to contribute peptides to MHC class II molecules. Also loading of ZPS to MHC class II molecules is enhanced by TLR signalling by inducing ZPS processing into the optimal length by deamination for MHC II binding. Furthermore, MHC II-peptide complexes and likely also MHC II-ZPS complexes are efficiently transported to the plasmamembrane via tubules emanating from the MIIC and the half life of MHC II-peptide complexes presented at the cell surface are increased upon TLR-ligand engagement. Next to the stimulation of antigen presentation that is restricted to the confined compartment that contain both the antigen and TLR ligand, TLR signalling also stimulates general antigen presentation by inducing secretion of cytokines that upregulates the capability of cells to present antigens. Because IFNγ enhance both cross-presentation and classical MHC I pathway by inducing the generation of the immunoproteasome, specifically cross-presentation by relocating TAP to phagosomes, and also classical MHC II pathway by increasing synthesis of MHC class II

molecules, it seems that this TLR-mediated signalling is not favouring any specific antigen presentation pathway. However, Dectin-1 was shown to modulate TLR signalling towards cytokine expression. This could indicate that the interplay between different pathogen recognition receptors is modulating this general respone. Whether TLR signalling that modulates antigen processing in the confined compartment favours cross-presentation or presentation via MHC class II molecules is not known. Thus far it is demonstrated for TLR signalling to enhance the antigen processing for the classical MHC II pathway. Which requirements are essential to antigen processing for cross-presentation are not known. This is hampered by the amount of pathways proposed for cross-presentation with each their distinct compartments and requirements. For instance, it is possible that after delivering a certain antigen to the stable early endosome compartment, TLR-ligand engagement within the endosome is required to keep it away from the lysosomal pathway. A recent article describes that Irgm3 via lipid bodies formation inhibits phagosome maturation, without altering the rate of phagocytosis and affecting loading of exogenous peptides onto MHC class I molecules for cross-presentation [347]. This could indicate a mechanism responsible for the blockage towards the lysosomal pathway and favoring cross-presentation.

The second step towards full activation of T cells is presence of costimulatory molecules. When costimulatory molecules are absent during the engagement of MHC-peptide complex and TCR, signal 1 results in a state of non-responsiveness called clonal anergy. The presence of costimulatory molecules simultaneous with signal 1 allows clonal expansion. Moreover, it was demonstrated that TLR control of antigen presentation favours the presentation of microbial antigens within the context of T-lymphocyte costimulatory molecule expression, leading to full activation of T cells. In other words, TLR license the antigen to fully activate the T cell. Therefore it seems that TLRs influence antigen processing and presentation that is regulated by various uptake receptors, but that the absence of TLR-engagement results in tolerization of T cells instead of full activation. Indeed, Wang *et al* [345] showed that deficiency in TLR2 resulted in a lack of upregulation of costimulatory molecule B7-2 and an almost diminished T cell activation upon stimulation with PS-A. In addition, studies suggest that antigen uptake by CLRs alone can result in tolerance [348].

The last step towards a full and specific T cell response is the determination of differentiation state of the activated T cell. A variety of both membrane-incorporated and soluble factors influence the outcome of this process. Especially, cytokines that are secreted by the APC itself or other neighbouring cells determine the differentiation state of the activated T cell. As described, activation of a TLR by its relevant ligand rapidly ignites a complex intracellular signaling cascade that ultimately results in upregulation of inflammatory genes and production of proinflammatory cytokines and interferons. Most TLR ligands stimulate APCs to produce cytokines that initiate T_H1 responses (such as IL-12 and IL-18). For instance the ZPS PS-A induce IL12 expression in a TLR2-dependent pathway that is vital for driving differentiation towards Th1 cell developmental process. However, it is also recently described that CLRs may influence TLR-induced cytokine expression. Thereby showing that next to recognition bacterial-derived zwitterionic polysaccharides, also the bacterial-derived glycans are modulating the outcome for differentiation.
Since Sp1 from *S. pneumoniae* is not inducing secretion of IL12 in either macrophages or DCs, ZPSs do not induce similar immune responses. This indicates that ZPS-specific immune responses occur, perhaps relying on differential recognition by innate immune receptors. Moreover, ZPS-specific activated $CD4^+CD45Rb^{low}$ T cells or $CD8^+CD28^-$ T cells induce anti-inflammatory cytokine such as IL10, that prevent the Th1-mediated inflammatory immune responses in abscess formation and fibrosis.
Moreover, the recent finding that Dectin-1 signaling modulates TLR2-signaling to induce profound IL23 responses illustrates that CLRs not only function as uptake receptor but also signaling receptor. This finding clearly demonstrates that TLRs influence antigen processing and presentation regulated by CLRs as antigen uptake receptors, and CLRs on their turn may influence signaling pathways initiated by TLRs.

Collectively, these findings illustrate that the ultimate outcome is the result of a concordant action dictated by pathogen-associated molecular patterns that determine which set of innate receptors are engaged.

Signaling by the receptors is not a simple model in which the sum of receptor signaling determines the immune response. If we focus on DCs, since they are the most prominent in antigen presentation of all APCs, we see that DCs are equipped with different molecular platforms to detect both microbial and non-microbial endogenous factors. For instance, the family of Toll-like receptors (TLRs) are PRRs that are present primarily intracellularly. In contrast, Fc-receptors are incorporated in the plasmamembrane and recognize antigens at the DC surface. Since microbes are composed of multiple components that can be recognized by different host receptors, it is logic that multiple receptors are able to bind to the microbe and as previously discussed the interplay is crucial in determining the fate of the microbe. In other words, an integration of a variety of signals must occur to drive a proper immune response. Signals for activation of DCs can be derived from many sources, such as IFNγ from T cells, or other cytokines that can function in a paracrine or autocrine fashion, danger signals, CD40L stimulation from helper T cells or NK T cells, and microbial products. The expression and function of receptors recognizing these signals on DCs varies significantly between mice and humans and in different DC subsets [286]. For instance, the type I Interferon production induced by viral double-stranded RNA depends on RIG-I in convential DCs, whereas it depends on TLR3 in plasmacytoid DCs. Also important, DCs are exposed sequentially and stochastically different stimuli: the microbial products and inflammatory cytokines are encountered initially in peripheral tissues, whereas the CD40L stimulance occurs when the DC have reached the T cell area of secondary lymphoid organs. Since it was demonstrated that for cytokine production, TLR4 stimulation (via LPS) has to be sustained until a time point that coincides with the time of transcription of cytokine genes[349], it seems that also the duration of receptor stimulation is crucial in DC activation. In addition, different stimuli can cooperate and synergize in the induction of DC activation. An example for this is the modulation of TLR signaling by C-type lectin receptor signaling [293]. Also compartimentalization is crucial for receptor signaling, as indicated by TLR4 signaling that induces two pathways: the Toll-interleukin 1 receptor domain-containing adaptor protein (TIRAP)-MyD88 pathway that induces cytokines, and the Toll-receptor-associated molecule (TRAM)–Toll-receptor-associated activator of interferon (TRIF) pathway that induces IFN. Inhibiting TLR4 endocytosis disrupts the TRAM–TRIF pathway, and localization of TRAM to endosomes is necessary for TLR4 signaling [287]. Thus, TIRAP-MyD88 signaling is initiated by TLR4 at the plasma membrane, whereas TRAM–TRIF signaling is initiated by endocytosed TLR4. It is also demonstrated in literature that cytokine production by DCs is regulated not only by the combination of stimuli, but also by the order in which the stimuli are delivered over a defined period of time[350]. The IL12p70 production is enhanced by IFNγ only prior to TLR signaling, and the TLR synergy is more pronounced when TLR3 or 4 ligands are encountered before TLR8-induced signaling [350].

Collectively, the findings summed in this report clearly demonstrate that carbohydrates in both pure form and in association with other molecules are biologically significant biomolecules and research of them is thus far underappreciated. The discovery that carbohydrates can be recognized by T cells is important for the development of new generation glycoconjugate vaccines. Current-generation glycoconjugate vaccines are typically prepared by polysaccharide modification at multiple sites, with subsequent coupling to proteins randomly in a cluster form. As described before, there are some issues with the current glycoconjugate vaccines. The conjugation process induces structural heterogeneity of the batches. It is also possible that carrier-induced epitope suppression occurs and the major problem is that most current glycoconjugate vaccines do not induce the most efficient immunity. This is reflected in requirement of booster vaccines. Due to the described immunogenicity of ZPS, it is likely that ZPS-glycoconjugates induce higher T-cell and antibody responses to both carrier and polysaccharide, as compared to normal PS-glycoconjugates. Since a ZPS is a carbohydrate and an antigen at the same time,

ZPSs present the opportunity to investigate the precise mechanism of carbohydrate recognition and immunological consequences.

In addition, demonstrating that carbohydrates can be recognized by T cell receptors indicate that perhaps the sugar-moieties on glycans or glycolipids –either alone or in combination- can induce specific immune responses. As an example, perhaps the sugar moieties are essential in the recognition of glycolipids that are presented via the MHC-like molecule CD1 to NK T cells.

Since it was suggested that polysaccharide-based vaccines function in part by induction of protective $CD8^+$ T cell responses, further research into the contribution of bacterial-derived carbohydrates in antigen presentation via MHC I will be essential for optimal formulation of polysaccharide-based vaccines.

References

[1] Reith, W and Mach, B. (2001). THE BARE LYMPHOCYTE SYNDROME AND THE REGULATION OF MHC EXPRESSION. *Annu. Rev. Immunol.* 19, 331-73.

[2] Reith, W, LeibundGut-Landmann, S, and Waldburger, JM. (2005). Regulation of MHC class II gene expression by the class II transactivator. *Nat. Rev. Immunol.* 5, 793-806.

[3] Kindt, TJ, Goldsby, RA, Osborne, BA, and Kuby, J. (2007) *Immunology*.

[4] Karlsson, L, Surh, CD, Sprent, J, and Peterson, PA. (1991). A novel class II MHC molecule with unusual tissue distribution. *Nature* 351, 485-8.

[5] [Anonymous]. (1999). Complete sequence and gene map of a human major histocompatibility complex. The MHC sequencing consortium. *Nature* 401, 921-3.

[6] Koch, N, McLellan, AD, and Neumann, J. (2007). A revised model for invariant chain-mediated assembly of MHC class II peptide receptors. *Trends Biochem. Sci.* 32, 532-7.

[7] Cosson, P and Bonifacino, JS. (1992). Role of transmembrane domain interactions in the assembly of class II MHC molecules. *Science* 258, 659-62.

[8] Anderson, MS and Miller, J. (1992). Invariant chain can function as a chaperone protein for class II major histocompatibility complex molecules. *Proc. Natl. Acad. Sci. U. S. A.* 89, 2282-6.

[9] Romagnoli, P and Germain, RN. (1994). The CLIP region of invariant chain plays a critical role in regulating major histocompatibility complex class II folding, transport, and peptide occupancy. *J. Exp. Med.* 180, 1107-13.

[10] Bremnes, B, Madsen, T, Gedde-Dahl, M, and Bakke, O. (1994). An LI and ML motif in the cytoplasmic tail of the MHC-associated invariant chain mediate rapid internalization. *J. Cell. Sci.* 107 (Pt 7), 2021-32.

[11] Liu, SH, Marks, MS, and Brodsky, FM. (1998). A dominant-negative clathrin mutant differentially affects trafficking of molecules with distinct sorting motifs in the class II major histocompatibility complex (MHC) pathway. *J. Cell Biol.* 140, 1023-37.

[12] Ong, GL, Goldenberg, DM, Hansen, HJ, and Mattes, MJ. (1999). Cell surface expression and metabolism of major histocompatibility complex class II invariant chain (CD74) by diverse cell lines. *Immunology* 98, 296-302.

[13] Maric, MA, Taylor, MD, and Blum, JS. (1994). Endosomal aspartic proteinases are required for invariant-chain processing. *Proc. Natl. Acad. Sci. U. S. A.* 91, 2171-5.

[14] Neefjes, JJ and Ploegh, HL. (1992). Inhibition of endosomal proteolytic activity by leupeptin blocks surface expression of MHC class II molecules and their conversion to SDS resistance alpha beta heterodimers in endosomes. *EMBO J.* 11, 411-6.

[15] Riese, RJ, Wolf, PR, Bromme, D, Natkin, LR, Villadangos, JA, Ploegh, HL, and Chapman, HA. (1996). Essential role for cathepsin S in MHC class II-associated invariant chain processing and peptide loading. *Immunity* 4, 357-66.

[16] Riberdy, JM, Newcomb, JR, Surman, MJ, Barbosa, JA, and Cresswell, P. (1992). HLA-DR molecules from an antigen-processing mutant cell line are associated with invariant chain peptides.

Nature 360, 474-7.

[17] Sanderson, F, Kleijmeer, MJ, Kelly, A, Verwoerd, D, Tulp, A, Neefjes, JJ, Geuze, HJ, and Trowsdale, J. (1994). Accumulation of HLA-DM, a regulator of antigen presentation, in MHC class II compartments. *Science* 266, 1566-9.

[18] Fremont, DH, Crawford, F, Marrack, P, Hendrickson, WA, and Kappler, J. (1998). Crystal structure of mouse H2-M. *Immunity* 9, 385-93.

[19] Mosyak, L, Zaller, DM, and Wiley, DC. (1998). The structure of HLA-DM, the peptide exchange catalyst that loads antigen onto class II MHC molecules during antigen presentation. *Immunity* 9, 377-83.

[20] Denzin, LK and Cresswell, P. (1995). HLA-DM induces CLIP dissociation from MHC class II alpha beta dimers and facilitates peptide loading. *Cell* 82, 155-65.

[21] Denzin, LK, Hammond, C, and Cresswell, P. (1996). HLA-DM interactions with intermediates in HLA-DR maturation and a role for HLA-DM in stabilizing empty HLA-DR molecules. *J. Exp. Med.* 184, 2153-65.

[22] Sloan, VS, Cameron, P, Porter, G, Gammon, M, Amaya, M, Mellins, E, and Zaller, DM. (1995). Mediation by HLA-DM of dissociation of peptides from HLA-DR. *Nature* 375, 802-6.

[23] Servenius, B, Rask, L, and Peterson, PA. (1987). Class II genes of the human major histocompatibility complex. The DO beta gene is a divergent member of the class II beta gene family. *J. Biol. Chem.* 262, 8759-66.

[24] Marks, MS, Roche, PA, van Donselaar, E, Woodruff, L, Peters, PJ, and Bonifacino, JS. (1995). A lysosomal targeting signal in the cytoplasmic tail of the beta chain directs HLA-DM to MHC class II compartments. *J. Cell Biol.* 131, 351-69.

[25] Liljedahl, M, Kuwana, T, Fung-Leung, WP, Jackson, MR, Peterson, PA, and Karlsson, L. (1996). HLA-DO is a lysosomal resident which requires association with HLA-DM for efficient intracellular transport. *EMBO J.* 15, 4817-24.

[26] Douek, DC and Altmann, DM. (1997). HLA-DO is an intracellular class II molecule with distinctive thymic expression. *Int. Immunol.* 9, 355-64.

[27] Kropshofer, H, Hammerling, GJ, and Vogt, AB. (1999). The impact of the non-classical MHC proteins HLA-DM and HLA-DO on loading of MHC class II molecules. *Immunol. Rev.* 172, 267-78.

[28] Denzin, LK, Sant'Angelo, DB, Hammond, C, Surman, MJ, and Cresswell, P. (1997). Negative regulation by HLA-DO of MHC class II-restricted antigen processing. *Science* 278, 106-9.

[29] Busch, R, Rinderknecht, CH, Roh, S, Lee, AW, Harding, JJ, Burster, T, Hornell, TM, and Mellins, ED. (2005). Achieving stability through editing and chaperoning: regulation of MHC class II peptide binding and expression. *Immunol. Rev.* 207, 242-60.

[30] Kleijmeer, M, Ramm, G, Schuurhuis, D, Griffith, J, Rescigno, M, Ricciardi-Castagnoli, P, Rudensky, AY, Ossendorp, F, Melief, CJ, Stoorvogel, W, and Geuze, HJ. (2001). Reorganization of multivesicular bodies regulates MHC class II antigen presentation by dendritic cells. *J. Cell Biol.* 155, 53-63.

[31] Barois, N, de Saint-Vis, B, Lebecque, S, Geuze, HJ, and Kleijmeer, MJ. (2002). MHC class II compartments in human dendritic cells undergo profound structural changes upon activation. *Traffic* 3,

894-905.

[32] Boes, M, Bertho, N, Cerny, J, Op den Brouw, M, Kirchhausen, T, and Ploegh, H. (2003). T cells induce extended class II MHC compartments in dendritic cells in a Toll-like receptor-dependent manner. *J. Immunol.* 171, 4081-8.

[33] Boes, M, Cerny, J, Massol, R, Op den Brouw, M, Kirchhausen, T, Chen, J, and Ploegh, HL. (2002). T-cell engagement of dendritic cells rapidly rearranges MHC class II transport. *Nature* 418, 983-8.

[34] Zhong, G, Romagnoli, P, and Germain, RN. (1997). Related leucine-based cytoplasmic targeting signals in invariant chain and major histocompatibility complex class II molecules control endocytic presentation of distinct determinants in a single protein. *J. Exp. Med.* 185, 429-38.

[35] van Niel, G, Wubbolts, R, Ten Broeke, T, Buschow, SI, Ossendorp, FA, Melief, CJ, Raposo, G, van Balkom, BW, and Stoorvogel, W. (2006). Dendritic cells regulate exposure of MHC class II at their plasma membrane by oligoubiquitination. *Immunity* 25, 885-94.

[36] Matsuki, Y, Ohmura-Hoshino, M, Goto, E, Aoki, M, Mito-Yoshida, M, Uematsu, M, Hasegawa, T, Koseki, H, Ohara, O, Nakayama, M, Toyooka, K, Matsuoka, K, Hotta, H, Yamamoto, A, and Ishido, S. (2007). Novel regulation of MHC class II function in B cells. *EMBO J.* 26, 846-54.

[37] Matsuki, Y, Ohmura-Hoshino, M, Goto, E, Aoki, M, Mito-Yoshida, M, Uematsu, M, Hasegawa, T, Koseki, H, Ohara, O, Nakayama, M, Toyooka, K, Matsuoka, K, Hotta, H, Yamamoto, A, and Ishido, S. (2007). Novel regulation of MHC class II function in B cells. *EMBO J.* 26, 846-54.

[38] De Gassart, A, Camosseto, V, Thibodeau, J, Ceppi, M, Catalan, N, Pierre, P, and Gatti, E. (2008). MHC class II stabilization at the surface of human dendritic cells is the result of maturation-dependent MARCH I down-regulation. *Proc. Natl. Acad. Sci. U. S. A.* 105, 3491-6.

[39] Scholz, C and Tampe, R. (2009). The peptide-loading complex--antigen translocation and MHC class I loading. *Biol. Chem.* 390, 783-94.

[40] Borissenko, L and Groll, M. (2007). Diversity of proteasomal missions: fine tuning of the immune response. *Biol. Chem.* 388, 947-55.

[41] Groll, M and Huber, R. (2005). Purification, crystallization, and X-ray analysis of the yeast 20S proteasome. *Methods Enzymol.* 398, 329-36.

[42] Nussbaum, AK, Dick, TP, Keilholz, W, Schirle, M, Stevanovic, S, Dietz, K, Heinemeyer, W, Groll, M, Wolf, DH, Huber, R, Rammensee, HG, and Schild, H. (1998). Cleavage motifs of the yeast 20S proteasome beta subunits deduced from digests of enolase 1. *Proc. Natl. Acad. Sci. U. S. A.* 95, 12504-9.

[43] Jariel-Encontre, I, Bossis, G, and Piechaczyk, M. (2008). Ubiquitin-independent degradation of proteins by the proteasome. *Biochim. Biophys. Acta* 1786, 153-77.

[44] Hanada, K, Yewdell, JW, and Yang, JC. (2004). Immune recognition of a human renal cancer antigen through post-translational protein splicing. *Nature* 427, 252-6.

[45] Vigneron, N, Stroobant, V, Chapiro, J, Ooms, A, Degiovanni, G, Morel, S, van der Bruggen, P, Boon, T, and Van den Eynde, BJ. (2004). An antigenic peptide produced by peptide splicing in the proteasome. *Science* 304, 587-90.

[46] Groettrup, M, Soza, A, Eggers, M, Kuehn, L, Dick, TP, Schild, H, Rammensee, HG, Koszinowski, UH, and Kloetzel, PM. (1996). A role for the proteasome regulator PA28alpha in antigen presentation. *Nature* 381, 166-8.

[47] Stohwasser, R, Salzmann, U, Giesebrecht, J, Kloetzel, PM, and Holzhutter, HG. (2000). Kinetic evidences for facilitation of peptide channelling by the proteasome activator PA28. *Eur. J. Biochem.* 267, 6221-30.

[48] Hill, CP, Masters, EI, and Whitby, FG. (2002). The 11S regulators of 20S proteasome activity. *Curr. Top. Microbiol. Immunol.* 268, 73-89.

[49] Yao, T and Cohen, RE. (1999). Giant proteases: beyond the proteasome. *Curr. Biol.* 9, R551-3.

[50] Kelly, A, Powis, SH, Kerr, LA, Mockridge, I, Elliott, T, Bastin, J, Uchanska-Ziegler, B, Ziegler, A, Trowsdale, J, and Townsend, A. (1992). Assembly and function of the two ABC transporter proteins encoded in the human major histocompatibility complex. *Nature* 355, 641-4.

[51] Ackerman, AL and Cresswell, P. (2003). Regulation of MHC class I transport in human dendritic cells and the dendritic-like cell line KG-1. *J. Immunol.* 170, 4178-88.

[52] Kawahara, M, York, IA, Hearn, A, Farfan, D, and Rock, KL. (2009). Analysis of the role of tripeptidyl peptidase II in MHC class I antigen presentation in vivo. *J. Immunol.* 183, 6069-77.

[53] Evnouchidou, I, Papakyriakou, A, and Stratikos, E. (2009). A new role for Zn(II) aminopeptidases: antigenic peptide generation and destruction. *Curr. Pharm. Des.* 15, 3656-70.

[54] Saveanu, L, Carroll, O, Lindo, V, Del Val, M, Lopez, D, Lepelletier, Y, Greer, F, Schomburg, L, Fruci, D, Niedermann, G, and van Endert, PM. (2005). Concerted peptide trimming by human ERAP1 and ERAP2 aminopeptidase complexes in the endoplasmic reticulum. *Nat. Immunol.* 6, 689-97.

[55] Zhang, Q, Tector, M, and Salter, RD. (1995). Calnexin recognizes carbohydrate and protein determinants of class I major histocompatibility complex molecules. *J. Biol. Chem.* 270, 3944-8.

[56] Ellgaard, L and Frickel, EM. (2003). Calnexin, calreticulin, and ERp57: teammates in glycoprotein folding. *Cell Biochem. Biophys.* 39, 223-47.

[57] Antoniou, AN, Santos, SG, Campbell, EC, Lynch, S, Arosa, FA, and Powis, SJ. (2007). ERp57 interacts with conserved cysteine residues in the MHC class I peptide-binding groove. *FEBS Lett.* 581, 1988-92.

[58] Lederkremer, GZ. (2009). Glycoprotein folding, quality control and ER-associated degradation. *Curr. Opin. Struct. Biol.* 19, 515-23.

[59] Hoseki, J, Ushioda, R, and Nagata, K. (2009). Mechanism and components of endoplasmic reticulum-associated degradation. *J. Biochem.* .

[60] Elliott, T and Williams, A. (2005). The optimization of peptide cargo bound to MHC class I molecules by the peptide-loading complex. *Immunol. Rev.* 207, 89-99.

[61] Uebel, S, Meyer, TH, Kraas, W, Kienle, S, Jung, G, Wiesmuller, KH, and Tampe, R. (1995). Requirements for peptide binding to the human transporter associated with antigen processing revealed by peptide scans and complex peptide libraries. *J. Biol. Chem.* 270, 18512-6.

[62] Knittler, MR, Alberts, P, Deverson, EV, and Howard, JC. (1999). Nucleotide binding by TAP mediates association with peptide and release of assembled MHC class I molecules. *Curr. Biol.* 9,

999-1008.

[63] Abe, F, Van Prooyen, N, Ladasky, JJ, and Edidin, M. (2009). Interaction of Bap31 and MHC class I molecules and their traffic out of the endoplasmic reticulum. *J. Immunol.* 182, 4776-83.

[64] Ladasky, JJ, Boyle, S, Seth, M, Li, H, Pentcheva, T, Abe, F, Steinberg, SJ, and Edidin, M. (2006). Bap31 enhances the endoplasmic reticulum export and quality control of human class I MHC molecules. *J. Immunol.* 177, 6172-81.

[65] Greenberg, ME, Iafrate, AJ, and Skowronski, J. (1998). The SH3 domain-binding surface and an acidic motif in HIV-1 Nef regulate trafficking of class I MHC complexes. *EMBO J.* 17, 2777-89.

[66] Brown, FD, Rozelle, AL, Yin, HL, Balla, T, and Donaldson, JG. (2001). Phosphatidylinositol 4,5-bisphosphate and Arf6-regulated membrane traffic. *J. Cell Biol.* 154, 1007-17.

[67] Caplan, S, Naslavsky, N, Hartnell, LM, Lodge, R, Polishchuk, RS, Donaldson, JG, and Bonifacino, JS. (2002). A tubular EHD1-containing compartment involved in the recycling of major histocompatibility complex class I molecules to the plasma membrane. *EMBO J.* 21, 2557-67.

[68] Naslavsky, N, Boehm, M, Backlund, PS,Jr, and Caplan, S. (2004). Rabenosyn-5 and EHD1 interact and sequentially regulate protein recycling to the plasma membrane. *Mol. Biol. Cell* 15, 2410-22.

[69] Weigert, R, Yeung, AC, Li, J, and Donaldson, JG. (2004). Rab22a regulates the recycling of membrane proteins internalized independently of clathrin. *Mol. Biol. Cell* 15, 3758-70.

[70] Massol, RH, Larsen, JE, and Kirchhausen, T. (2005). Possible role of deep tubular invaginations of the plasma membrane in MHC-I trafficking. *Exp. Cell Res.* 306, 142-9.

[71] Rock, KL, Gamble, S, and Rothstein, L. (1990). Presentation of exogenous antigen with class I major histocompatibility complex molecules. *Science* 249, 918-21.

[72] Carbone, FR and Bevan, MJ. (1990). Class I-restricted processing and presentation of exogenous cell-associated antigen in vivo. *J. Exp. Med.* 171, 377-87.

[73] Kovacsovics-Bankowski, M, Clark, K, Benacerraf, B, and Rock, KL. (1993). Efficient major histocompatibility complex class I presentation of exogenous antigen upon phagocytosis by macrophages. *Proc. Natl. Acad. Sci. U. S. A.* 90, 4942-6.

[74] Shen, Z, Reznikoff, G, Dranoff, G, and Rock, KL. (1997). Cloned dendritic cells can present exogenous antigens on both MHC class I and class II molecules. *J. Immunol.* 158, 2723-30.

[75] Heit, A, Huster, KM, Schmitz, F, Schiemann, M, Busch, DH, and Wagner, H. (2004). CpG-DNA aided cross-priming by cross-presenting B cells. *J. Immunol.* 172, 1501-7.

[76] Hon, H, Oran, A, Brocker, T, and Jacob, J. (2005). B lymphocytes participate in cross-presentation of antigen following gene gun vaccination. *J. Immunol.* 174, 5233-42.

[77] Tvinnereim, AR, Hamilton, SE, and Harty, JT. (2004). Neutrophil involvement in cross-priming CD8+ T cell responses to bacterial antigens. *J. Immunol.* 173, 1994-2002.

[78] Beauvillain, C, Delneste, Y, Scotet, M, Peres, A, Gascan, H, Guermonprez, P, Barnaba, V, and Jeannin, P. (2007). Neutrophils efficiently cross-prime naive T cells in vivo. *Blood* 110, 2965-73.

[79] Bagai, R, Valujskikh, A, Canaday, DH, Bailey, E, Lalli, PN, Harding, CV, and Heeger, PS. (2005). Mouse endothelial cells cross-present lymphocyte-derived antigen on class I MHC via a TAP1- and proteasome-dependent pathway. *J. Immunol.* 174, 7711-5.

[80] Jung, S, Unutmaz, D, Wong, P, Sano, G, De los Santos, K, Sparwasser, T, Wu, S, Vuthoori, S, Ko, K, Zavala, F, Pamer, EG, Littman, DR, and Lang, RA. (2002). In vivo depletion of CD11c(+) dendritic cells abrogates priming of CD8(+) T cells by exogenous cell-associated antigens. *Immunity* 17, 211-20.

[81] Storni, T and Bachmann, MF. (2004). Loading of MHC class I and II presentation pathways by exogenous antigens: a quantitative in vivo comparison. *J. Immunol.* 172, 6129-35.

[82] Savina, A, Peres, A, Cebrian, I, Carmo, N, Moita, C, Hacohen, N, Moita, LF, and Amigorena, S. (2009). The small GTPase Rac2 controls phagosomal alkalinization and antigen crosspresentation selectively in CD8(+) dendritic cells. *Immunity* 30, 544-55.

[83] Delamarre, L, Pack, M, Chang, H, Mellman, I, and Trombetta, ES. (2005). Differential lysosomal proteolysis in antigen-presenting cells determines antigen fate. *Science* 307, 1630-4.

[84] Trombetta, ES, Ebersold, M, Garrett, W, Pypaert, M, and Mellman, I. (2003). Activation of lysosomal function during dendritic cell maturation. *Science* 299, 1400-3.

[85] Hotta, C, Fujimaki, H, Yoshinari, M, Nakazawa, M, and Minami, M. (2006). The delivery of an antigen from the endocytic compartment into the cytosol for cross-presentation is restricted to early immature dendritic cells. *Immunology* 117, 97-107.

[86] Belz, GT, Smith, CM, Eichner, D, Shortman, K, Karupiah, G, Carbone, FR, and Heath, WR. (2004). Cutting edge: conventional CD8 alpha+ dendritic cells are generally involved in priming CTL immunity to viruses. *J. Immunol.* 172, 1996-2000.

[87] Belz, GT, Behrens, GM, Smith, CM, Miller, JF, Jones, C, Lejon, K, Fathman, CG, Mueller, SN, Shortman, K, Carbone, FR, and Heath, WR. (2002). The CD8alpha(+) dendritic cell is responsible for inducing peripheral self-tolerance to tissue-associated antigens. *J. Exp. Med.* 196, 1099-104.

[88] Pooley, JL, Heath, WR, and Shortman, K. (2001). Cutting edge: intravenous soluble antigen is presented to CD4 T cells by CD8- dendritic cells, but cross-presented to CD8 T cells by CD8+ dendritic cells. *J. Immunol.* 166, 5327-30.

[89] den Haan, JM and Bevan, MJ. (2002). Constitutive versus activation-dependent cross-presentation of immune complexes by CD8(+) and CD8(-) dendritic cells in vivo. *J. Exp. Med.* 196, 817-27.

[90] Khor, B and Makar, RS. (2008). Toward a molecular explanation for cross-presentation of antigens to the immune system. *Transfus. Med. Rev.* 22, 188-201.

[91] Pulendran, B, Lingappa, J, Kennedy, MK, Smith, J, Teepe, M, Rudensky, A, Maliszewski, CR, and Maraskovsky, E. (1997). Developmental pathways of dendritic cells in vivo: distinct function, phenotype, and localization of dendritic cell subsets in FLT3 ligand-treated mice. *J. Immunol.* 159, 2222-31.

[92] Allan, RS, Smith, CM, Belz, GT, van Lint, AL, Wakim, LM, Heath, WR, and Carbone, FR. (2003). Epidermal viral immunity induced by CD8alpha+ dendritic cells but not by Langerhans cells. *Science* 301, 1925-8.

[93] Allan, RS, Waithman, J, Bedoui, S, Jones, CM, Villadangos, JA, Zhan, Y, Lew, AM, Shortman, K, Heath, WR, and Carbone, FR. (2006). Migratory dendritic cells transfer antigen to a lymph node-resident dendritic cell population for efficient CTL priming. *Immunity* 25, 153-62.

[94] Thery, C, Zitvogel, L, and Amigorena, S. (2002). Exosomes: composition, biogenesis and function. *Nat. Rev. Immunol.* 2, 569-79.

[95] Fleeton, MN, Contractor, N, Leon, F, Wetzel, JD, Dermody, TS, and Kelsall, BL. (2004). Peyer's patch dendritic cells process viral antigen from apoptotic epithelial cells in the intestine of reovirus-infected mice. *J. Exp. Med.* 200, 235-45.

[96] van Montfoort, N, Camps, MG, Khan, S, Filippov, DV, Weterings, JJ, Griffith, JM, Geuze, HJ, van Hall, T, Verbeek, JS, Melief, CJ, and Ossendorp, F. (2009). Antigen storage compartments in mature dendritic cells facilitate prolonged cytotoxic T lymphocyte cross-priming capacity. *Proc. Natl. Acad. Sci. U. S. A.* 106, 6730-5.

[97] Kovacsovics-Bankowski, M and Rock, KL. (1995). A phagosome-to-cytosol pathway for exogenous antigens presented on MHC class I molecules. *Science* 267, 243-6.

[98] Burgdorf, S, Lukacs-Kornek, V, and Kurts, C. (2006). The mannose receptor mediates uptake of soluble but not of cell-associated antigen for cross-presentation. *J. Immunol.* 176, 6770-6.

[99] Song, R and Harding, CV. (1996). Roles of proteasomes, transporter for antigen presentation (TAP), and beta 2-microglobulin in the processing of bacterial or particulate antigens via an alternate class I MHC processing pathway. *J. Immunol.* 156, 4182-90.

[100] Shen, L, Sigal, LJ, Boes, M, and Rock, KL. (2004). Important role of cathepsin S in generating peptides for TAP-independent MHC class I crosspresentation in vivo. *Immunity* 21, 155-65.

[101] Accapezzato, D, Visco, V, Francavilla, V, Molette, C, Donato, T, Paroli, M, Mondelli, MU, Doria, M, Torrisi, MR, and Barnaba, V. (2005). Chloroquine enhances human CD8+ T cell responses against soluble antigens in vivo. *J. Exp. Med.* 202, 817-28.

[102] Harding, CV and Song, R. (1994). Phagocytic processing of exogenous particulate antigens by macrophages for presentation by class I MHC molecules. *J. Immunol.* 153, 4925-33.

[103] Kovacsovics-Bankowski, M, Clark, K, Benacerraf, B, and Rock, KL. (1993). Efficient major histocompatibility complex class I presentation of exogenous antigen upon phagocytosis by macrophages. *Proc. Natl. Acad. Sci. U. S. A.* 90, 4942-6.

[104] Rodriguez, A, Regnault, A, Kleijmeer, M, Ricciardi-Castagnoli, P, and Amigorena, S. (1999). Selective transport of internalized antigens to the cytosol for MHC class I presentation in dendritic cells. *Nat. Cell Biol.* 1, 362-8.

[105] Ackerman, AL, Giodini, A, and Cresswell, P. (2006). A role for the endoplasmic reticulum protein retrotranslocation machinery during crosspresentation by dendritic cells. *Immunity* 25, 607-17.

[106] Brossart, P and Bevan, MJ. (1997). Presentation of exogenous protein antigens on major histocompatibility complex class I molecules by dendritic cells: pathway of presentation and regulation by cytokines. *Blood* 90, 1594-9.

[107] Bougneres, L, Helft, J, Tiwari, S, Vargas, P, Chang, BH, Chan, L, Campisi, L, Lauvau, G, Hugues, S, Kumar, P, Kamphorst, AO, Dumenil, AM, Nussenzweig, M, MacMicking, JD, Amigorena, S, and Guermonprez, P. (2009). A role for lipid bodies in the cross-presentation of phagocytosed

antigens by MHC class I in dendritic cells. *Immunity* 31, 232-44.

[108] Imai, J, Hasegawa, H, Maruya, M, Koyasu, S, and Yahara, I. (2005). Exogenous antigens are processed through the endoplasmic reticulum-associated degradation (ERAD) in cross-presentation by dendritic cells. *Int. Immunol.* 17, 45-53.

[109] Gagnon, E, Duclos, S, Rondeau, C, Chevet, E, Cameron, PH, Steele-Mortimer, O, Paiement, J, Bergeron, JJ, and Desjardins, M. (2002). Endoplasmic reticulum-mediated phagocytosis is a mechanism of entry into macrophages. *Cell* 110, 119-31.

[110] Houde, M, Bertholet, S, Gagnon, E, Brunet, S, Goyette, G, Laplante, A, Princiotta, MF, Thibault, P, Sacks, D, and Desjardins, M. (2003). Phagosomes are competent organelles for antigen cross-presentation. *Nature* 425, 402-6.

[111] Guermonprez, P, Saveanu, L, Kleijmeer, M, Davoust, J, Van Endert, P, and Amigorena, S. (2003). ER-phagosome fusion defines an MHC class I cross-presentation compartment in dendritic cells. *Nature* 425, 397-402.

[112] Yewdell, JW and Haeryfar, SM. (2005). Understanding presentation of viral antigens to CD8+ T cells in vivo: the key to rational vaccine design. *Annu. Rev. Immunol.* 23, 651-82.

[113] Saveanu, L, Carroll, O, Weimershaus, M, Guermonprez, P, Firat, E, Lindo, V, Greer, F, Davoust, J, Kratzer, R, Keller, SR, Niedermann, G, and van Endert, P. (2009). IRAP identifies an endosomal compartment required for MHC class I cross-presentation. *Science* 325, 213-7.

[114] Burgdorf, S, Kautz, A, Bohnert, V, Knolle, PA, and Kurts, C. (2007). Distinct pathways of antigen uptake and intracellular routing in CD4 and CD8 T cell activation. *Science* 316, 612-6.

[115] Burgdorf, S, Scholz, C, Kautz, A, Tampe, R, and Kurts, C. (2008). Spatial and mechanistic separation of cross-presentation and endogenous antigen presentation. *Nat. Immunol.* 9, 558-66.

[116] Touret, N, Paroutis, P, Terebiznik, M, Harrison, RE, Trombetta, S, Pypaert, M, Chow, A, Jiang, A, Shaw, J, Yip, C, Moore, HP, van der Wel, N, Houben, D, Peters, PJ, de Chastellier, C, Mellman, I, and Grinstein, S. (2005). Quantitative and dynamic assessment of the contribution of the ER to phagosome formation. *Cell* 123, 157-70.

[117] Groothuis, TA and Neefjes, J. (2005). The many roads to cross-presentation. *J. Exp. Med.* 202, 1313-8.

[118] Pfeifer, JD, Wick, MJ, Roberts, RL, Findlay, K, Normark, SJ, and Harding, CV. (1993). Phagocytic processing of bacterial antigens for class I MHC presentation to T cells. *Nature* 361, 359-62.

[119] Campbell, DJ, Serwold, T, and Shastri, N. (2000). Bacterial proteins can be processed by macrophages in a transporter associated with antigen processing-independent, cysteine protease-dependent manner for presentation by MHC class I molecules. *J. Immunol.* 164, 168-75.

[120] Wick, MJ and Pfeifer, JD. (1996). Major histocompatibility complex class I presentation of ovalbumin peptide 257-264 from exogenous sources: protein context influences the degree of TAP-independent presentation. *Eur. J. Immunol.* 26, 2790-9.

[121] Bachmann, MF, Oxenius, A, Pircher, H, Hengartner, H, Ashton-Richardt, PA, Tonegawa, S, and Zinkernagel, RM. (1995). TAP1-independent loading of class I molecules by exogenous viral proteins. *Eur. J. Immunol.* 25, 1739-43.

[122] Stober, D, Trobonjaca, Z, Reimann, J, and Schirmbeck, R. (2002). Dendritic cells pulsed with exogenous hepatitis B surface antigen particles efficiently present epitopes to MHC class I-restricted cytotoxic T cells. *Eur. J. Immunol.* 32, 1099-108.

[123] Norbury, CC, Princiotta, MF, Bacik, I, Brutkiewicz, RR, Wood, P, Elliott, T, Bennink, JR, and Yewdell, JW. (2001). Multiple antigen-specific processing pathways for activating naive CD8+ T cells in vivo. *J. Immunol.* 166, 4355-62.

[124] Ruedl, C, Storni, T, Lechner, F, Bachi, T, and Bachmann, MF. (2002). Cross-presentation of virus-like particles by skin-derived CD8(-) dendritic cells: a dispensable role for TAP. *Eur. J. Immunol.* 32, 818-25.

[125] Gromme, M, Uytdehaag, FG, Janssen, H, Calafat, J, van Binnendijk, RS, Kenter, MJ, Tulp, A, Verwoerd, D, and Neefjes, J. (1999). Recycling MHC class I molecules and endosomal peptide loading. *Proc. Natl. Acad. Sci. U. S. A.* 96, 10326-31.

[126] Zou, L, Zhou, J, Zhang, J, Li, J, Liu, N, Chai, L, Li, N, Liu, T, Li, L, Xie, Z, Liu, H, Wan, Y, and Wu, Y. (2009). The GTPase Rab3b/3c-positive recycling vesicles are involved in cross-presentation in dendritic cells. *Proc. Natl. Acad. Sci. U. S. A.* 106, 15801-6.

[127] Kim, SH, Visser, A, Cruijsen, C, van der Velden, AW, and Boes, M. (2008). Recruitment of Rab27a to phagosomes controls microbial antigen cross-presentation by dendritic cells. *Infect. Immun.* 76, 5373-80.

[128] Henrichsen, J. (1995). Six newly recognized types of Streptococcus pneumoniae. *J. Clin. Microbiol.* 33, 2759-62.

[129] Jennings, HJ. (1983). Capsular polysaccharides as human vaccines. *Adv. Carbohydr. Chem. Biochem.* 41, 155-208.

[130] Lucas, AH, Apicella, MA, and Taylor, CE. (2005). Carbohydrate moieties as vaccine candidates. *Clin. Infect. Dis.* 41, 705-12.

[131] Szymanski, CM and Wren, BW. (2005). Protein glycosylation in bacterial mucosal pathogens. *Nat. Rev. Microbiol.* 3, 225-37.

[132] Feizi, T and Chai, W. (2004). Oligosaccharide microarrays to decipher the glyco code. *Nat. Rev. Mol. Cell Biol.* 5, 582-8.

[133] Wildt, S and Gerngross, TU. (2005). The humanization of N-glycosylation pathways in yeast. *Nat. Rev. Microbiol.* 3, 119-28.

[134] Lovering, AL, de Castro, LH, Lim, D, and Strynadka, NC. (2007). Structural insight into the transglycosylation step of bacterial cell-wall biosynthesis. *Science* 315, 1402-5.

[135] Garcia-Vallejo, JJ and van Kooyk, Y. (2009). Endogenous ligands for C-type lectin receptors: the true regulators of immune homeostasis. *Immunol. Rev.* 230, 22-37.

[136] Feinberg, H, Guo, Y, Mitchell, DA, Drickamer, K, and Weis, WI. (2005). Extended neck regions stabilize tetramers of the receptors DC-SIGN and DC-SIGNR. *J. Biol. Chem.* 280, 1327-35.

[137] Guo, Y, Feinberg, H, Conroy, E, Mitchell, DA, Alvarez, R, Blixt, O, Taylor, ME, Weis, WI, and Drickamer, K. (2004). Structural basis for distinct ligand-binding and targeting properties of the receptors DC-SIGN and DC-SIGNR. *Nat. Struct. Mol. Biol.* 11, 591-8.

[138] Dam, TK, Gerken, TA, Cavada, BS, Nascimento, KS, Moura, TR, and Brewer, CF. (2007). Binding studies of alpha-GalNAc-specific lectins to the alpha-GalNAc (Tn-antigen) form of porcine submaxillary mucin and its smaller fragments. *J. Biol. Chem.* 282, 28256-63.

[139] Dam, TK and Brewer, CF. (2009). Lectins as Pattern Recognition Molecules: The Effects of Epitope Density in Innate Immunity#. *Glycobiology* .

[140] Brewer, CF, Miceli, MC, and Baum, LG. (2002). Clusters, bundles, arrays and lattices: novel mechanisms for lectin-saccharide-mediated cellular interactions. *Curr. Opin. Struct. Biol.* 12, 616-23.

[141] Marth, JD and Grewal, PK. (2008). Mammalian glycosylation in immunity. *Nat. Rev. Immunol.* 8, 874-87.

[142] van Kooyk, Y and Rabinovich, GA. (2008). Protein-glycan interactions in the control of innate and adaptive immune responses. *Nat. Immunol.* 9, 593-601.

[143] Vasta, GR. (2009). Roles of galectins in infection. *Nat. Rev. Microbiol.* 7, 424-38.

[144] Dam, TK and Brewer, CF. (2008). Effects of clustered epitopes in multivalent ligand-receptor interactions. *Biochemistry* 47, 8470-6.

[145] Weis, WI, Taylor, ME, and Drickamer, K. (1998). The C-type lectin superfamily in the immune system. *Immunol. Rev.* 163, 19-34.

[146] Rando, RR, Orr, GA, and Bangerter, FW. (1979). Threshold effects on the concanavalin A-mediated agglutination of modified erythrocytes. *J. Biol. Chem.* 254, 8318-23.

[147] Newman, BA and Kabat, EA. (1985). An immunochemical study of the combining site specificities of C57BL/6J monoclonal antibodies to alpha (1----6)-linked dextran B512. *J. Immunol.* 135, 1220-31.

[148] Weis, WI and Drickamer, K. (1996). Structural basis of lectin-carbohydrate recognition. *Annu. Rev. Biochem.* 65, 441-73.

[149] James, LC, Roversi, P, and Tawfik, DS. (2003). Antibody multispecificity mediated by conformational diversity. *Science* 299, 1362-7.

[150] Wessels, MR, Paoletti, LC, Rodewald, AK, Michon, F, DiFabio, J, Jennings, HJ, and Kasper, DL. (1993). Stimulation of protective antibodies against type Ia and Ib group B streptococci by a type Ia polysaccharide-tetanus toxoid conjugate vaccine. *Infect. Immun.* 61, 4760-6.

[151] Kogelberg, H and Feizi, T. (2001). New structural insights into lectin-type proteins of the immune system. *Curr. Opin. Struct. Biol.* 11, 635-43.

[152] Gupta, G and Surolia, A. (2007). Collectins: sentinels of innate immunity. *Bioessays* 29, 452-64.

[153] Suresh, MV, Singh, SK, Ferguson, DA,Jr, and Agrawal, A. (2006). Role of the property of C-reactive protein to activate the classical pathway of complement in protecting mice from pneumococcal infection. *J. Immunol.* 176, 4369-74.

[154] Mold, C, Gresham, HD, and Du Clos, TW. (2001). Serum amyloid P component and C-reactive protein mediate phagocytosis through murine Fc gamma Rs. *J. Immunol.* 166, 1200-5.

[155] Fujita, T, Matsushita, M, and Endo, Y. (2004). The lectin-complement pathway--its role in innate immunity and evolution. *Immunol. Rev.* 198, 185-202.

[156] Liu, Y, Endo, Y, Homma, S, Kanno, K, Yaginuma, H, and Fujita, T. (2005). Ficolin A and ficolin B are expressed in distinct ontogenic patterns and cell types in the mouse. *Mol. Immunol.* 42, 1265-73.

[157] Endo, Y, Nakazawa, N, Liu, Y, Iwaki, D, Takahashi, M, Fujita, T, Nakata, M, and Matsushita, M. (2005). Carbohydrate-binding specificities of mouse ficolin A, a splicing variant of ficolin A and ficolin B and their complex formation with MASP-2 and sMAP. *Immunogenetics* 57, 837-44.

[158] Hoffmann, JA and Reichhart, JM. (2002). Drosophila innate immunity: an evolutionary perspective. *Nat. Immunol.* 3, 121-6.

[159] Lemaitre, B, Nicolas, E, Michaut, L, Reichhart, JM, and Hoffmann, JA. (1996). The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults. *Cell* 86, 973-83.

[160] Rock, FL, Hardiman, G, Timans, JC, Kastelein, RA, and Bazan, JF. (1998). A family of human receptors structurally related to Drosophila Toll. *Proc. Natl. Acad. Sci. U. S. A.* 95, 588-93.

[161] Janeway, CA,Jr and Medzhitov, R. (2002). Innate immune recognition. *Annu. Rev. Immunol.* 20, 197-216.

[162] Campos, MA, Almeida, IC, Takeuchi, O, Akira, S, Valente, EP, Procopio, DO, Travassos, LR, Smith, JA, Golenbock, DT, and Gazzinelli, RT. (2001). Activation of Toll-like receptor-2 by glycosylphosphatidylinositol anchors from a protozoan parasite. *J. Immunol.* 167, 416-23.

[163] Li, M, Carpio, DF, Zheng, Y, Bruzzo, P, Singh, V, Ouaaz, F, Medzhitov, RM, and Beg, AA. (2001). An essential role of the NF-kappa B/Toll-like receptor pathway in induction of inflammatory and tissue-repair gene expression by necrotic cells. *J. Immunol.* 166, 7128-35.

[164] Schleifer, KH and Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol. Rev.* 36, 407-77.

[165] Iwaki, D, Mitsuzawa, H, Murakami, S, Sano, H, Konishi, M, Akino, T, and Kuroki, Y. (2002). The extracellular toll-like receptor 2 domain directly binds peptidoglycan derived from Staphylococcus aureus. *J. Biol. Chem.* 277, 24315-20.

[166] Mitsuzawa, H, Wada, I, Sano, H, Iwaki, D, Murakami, S, Himi, T, Matsushima, N, and Kuroki, Y. (2001). Extracellular Toll-like receptor 2 region containing Ser40-Ile64 but not Cys30-Ser39 is critical for the recognition of Staphylococcus aureus peptidoglycan. *J. Biol. Chem.* 276, 41350-6.

[167] Shimazu, R, Akashi, S, Ogata, H, Nagai, Y, Fukudome, K, Miyake, K, and Kimoto, M. (1999). MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. *J. Exp. Med.* 189, 1777-82.

[168] Viriyakosol, S, Tobias, PS, Kitchens, RL, and Kirkland, TN. (2001). MD-2 binds to bacterial lipopolysaccharide. *J. Biol. Chem.* 276, 38044-51.

[169] Tissieres, P, Dunn-Siegrist, I, Schappi, M, Elson, G, Comte, R, Nobre, V, and Pugin, J. (2008). Soluble MD-2 is an acute-phase protein and an opsonin for Gram-negative bacteria. *Blood* 111, 2122-31.

[170] Erridge, C, Bennett-Guerrero, E, and Poxton, IR. (2002). Structure and function of lipopolysaccharides. *Microbes Infect.* 4, 837-51.

[171] Rietschel, ET, Brade, H, Brade, L, Brandenburg, K, Schade, U, Seydel, U, Zahringer, U, Galanos, C, Luderitz, O, and Westphal, O. (1987). Lipid A, the endotoxic center of bacterial lipopolysaccharides: relation of chemical structure to biological activity. *Prog. Clin. Biol. Res.* 231, 25-53.

[172] Rietschel, ET, Seydel, U, Zahringer, U, Schade, UF, Brade, L, Loppnow, H, Feist, W, Wang, MH, Ulmer, AJ, and Flad, HD. (1991). Bacterial endotoxin: molecular relationships between structure and activity. *Infect. Dis. Clin. North Am.* 5, 753-79.

[173] Kim, HM, Park, BS, Kim, JI, Kim, SE, Lee, J, Oh, SC, Enkhbayar, P, Matsushima, N, Lee, H, Yoo, OJ, and Lee, JO. (2007). Crystal structure of the TLR4-MD-2 complex with bound endotoxin antagonist Eritoran. *Cell* 130, 906-17.

[174] Visintin, A, Halmen, KA, Latz, E, Monks, BG, and Golenbock, DT. (2005). Pharmacological inhibition of endotoxin responses is achieved by targeting the TLR4 coreceptor, MD-2. *J. Immunol.* 175, 6465-72.

[175] Chakraborty, P, Ghosh, D, and Basu, MK. (2001). Modulation of macrophage mannose receptor affects the uptake of virulent and avirulent Leishmania donovani promastigotes. *J. Parasitol.* 87, 1023-7.

[176] Ezekowitz, RA, Williams, DJ, Koziel, H, Armstrong, MY, Warner, A, Richards, FF, and Rose, RM. (1991). Uptake of Pneumocystis carinii mediated by the macrophage mannose receptor. *Nature* 351, 155-8.

[177] Marodi, L, Korchak, HM, and Johnston, RB,Jr. (1991). Mechanisms of host defense against Candida species. I. Phagocytosis by monocytes and monocyte-derived macrophages. *J. Immunol.* 146, 2783-9.

[178] O'Riordan, DM, Standing, JE, and Limper, AH. (1995). Pneumocystis carinii glycoprotein A binds macrophage mannose receptors. *Infect. Immun.* 63, 779-84.

[179] Schlesinger, LS. (1993). Macrophage phagocytosis of virulent but not attenuated strains of Mycobacterium tuberculosis is mediated by mannose receptors in addition to complement receptors. *J. Immunol.* 150, 2920-30.

[180] Zamze, S, Martinez-Pomares, L, Jones, H, Taylor, PR, Stillion, RJ, Gordon, S, and Wong, SY. (2002). Recognition of bacterial capsular polysaccharides and lipopolysaccharides by the macrophage mannose receptor. *J. Biol. Chem.* 277, 41613-23.

[181] Geijtenbeek, TB, Krooshoop, DJ, Bleijs, DA, van Vliet, SJ, van Duijnhoven, GC, Grabovsky, V, Alon, R, Figdor, CG, and van Kooyk, Y. (2000). DC-SIGN-ICAM-2 interaction mediates dendritic cell trafficking. *Nat. Immunol.* 1, 353-7.

[182] Krutzik, SR, Tan, B, Li, H, Ochoa, MT, Liu, PT, Sharfstein, SE, Graeber, TG, Sieling, PA, Liu, YJ, Rea, TH, Bloom, BR, and Modlin, RL. (2005). TLR activation triggers the rapid differentiation of monocytes into macrophages and dendritic cells. *Nat. Med.* 11, 653-60.

[183] Lai, WK, Sun, PJ, Zhang, J, Jennings, A, Lalor, PF, Hubscher, S, McKeating, JA, and Adams, DH. (2006). Expression of DC-SIGN and DC-SIGNR on human sinusoidal endothelium: a role for capturing hepatitis C virus particles. *Am. J. Pathol.* 169, 200-8.

[184] Soilleux, EJ, Morris, LS, Leslie, G, Chehimi, J, Luo, Q, Levroney, E, Trowsdale, J, Montaner, LJ, Doms, RW, Weissman, D, Coleman, N, and Lee, B. (2002). Constitutive and induced expression of DC-SIGN on dendritic cell and macrophage subpopulations in situ and in vitro. *J. Leukoc. Biol.* 71, 445-57.

[185] Tailleux, L, Pham-Thi, N, Bergeron-Lafaurie, A, Herrmann, JL, Charles, P, Schwartz, O, Scheinmann, P, Lagrange, PH, de Blic, J, Tazi, A, Gicquel, B, and Neyrolles, O. (2005). DC-SIGN induction in alveolar macrophages defines privileged target host cells for mycobacteria in patients with tuberculosis. *PLoS Med.* 2, e381.

[186] Mitchell, DA, Fadden, AJ, and Drickamer, K. (2001). A novel mechanism of carbohydrate recognition by the C-type lectins DC-SIGN and DC-SIGNR. Subunit organization and binding to multivalent ligands. *J. Biol. Chem.* 276, 28939-45.

[187] Appelmelk, BJ, van Die, I, van Vliet, SJ, Vandenbroucke-Grauls, CM, Geijtenbeek, TB, and van Kooyk, Y. (2003). Cutting edge: carbohydrate profiling identifies new pathogens that interact with dendritic cell-specific ICAM-3-grabbing nonintegrin on dendritic cells. *J. Immunol.* 170, 1635-9.

[188] Cambi, A, Netea, MG, Mora-Montes, HM, Gow, NA, Hato, SV, Lowman, DW, Kullberg, BJ, Torensma, R, Williams, DL, and Figdor, CG. (2008). Dendritic cell interaction with Candida albicans critically depends on N-linked mannan. *J. Biol. Chem.* 283, 20590-9.

[189] Geijtenbeek, TB and van Kooyk, Y. (2003). DC-SIGN: a novel HIV receptor on DCs that mediates HIV-1 transmission. *Curr. Top. Microbiol. Immunol.* 276, 31-54.

[190] Serrano-Gomez, D, Dominguez-Soto, A, Ancochea, J, Jimenez-Heffernan, JA, Leal, JA, and Corbi, AL. (2004). Dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin mediates binding and internalization of Aspergillus fumigatus conidia by dendritic cells and macrophages. *J. Immunol.* 173, 5635-43.

[191] Tailleux, L, Schwartz, O, Herrmann, JL, Pivert, E, Jackson, M, Amara, A, Legres, L, Dreher, D, Nicod, LP, Gluckman, JC, Lagrange, PH, Gicquel, B, and Neyrolles, O. (2003). DC-SIGN is the major Mycobacterium tuberculosis receptor on human dendritic cells. *J. Exp. Med.* 197, 121-7.

[192] van Die, I, van Vliet, SJ, Nyame, AK, Cummings, RD, Bank, CM, Appelmelk, B, Geijtenbeek, TB, and van Kooyk, Y. (2003). The dendritic cell-specific C-type lectin DC-SIGN is a receptor for Schistosoma mansoni egg antigens and recognizes the glycan antigen Lewis x. *Glycobiology* 13, 471-8.

[193] Davies, J, Neth, O, Alton, E, Klein, N, and Turner, M. (2000). Differential binding of mannose-binding lectin to respiratory pathogens in cystic fibrosis. *Lancet* 355, 1885-6.

[194] Neth, O, Jack, DL, Dodds, AW, Holzel, H, Klein, NJ, and Turner, MW. (2000). Mannose-binding lectin binds to a range of clinically relevant microorganisms and promotes complement deposition. *Infect. Immun.* 68, 688-93.

[195] Tabona, P, Mellor, A, and Summerfield, JA. (1995). Mannose binding protein is involved in first-line host defence: evidence from transgenic mice. *Immunology* 85, 153-9.

[196] van Emmerik, LC, Kuijper, EJ, Fijen, CA, Dankert, J, and Thiel, S. (1994). Binding of mannan-binding protein to various bacterial pathogens of meningitis. *Clin. Exp. Immunol.* 97, 411-6.

[197] Hartshorn, KL, Crouch, E, White, MR, Colamussi, ML, Kakkanatt, A, Tauber, B, Shepherd, V, and Sastry, KN. (1998). Pulmonary surfactant proteins A and D enhance neutrophil uptake of bacteria.

Am. J. Physiol. 274, L958-69.

[198] Khubchandani, KR, Oberley, RE, and Snyder, JM. (2001). Effects of surfactant protein A and NaCl concentration on the uptake of Pseudomonas aeruginosa by THP-1 cells. *Am. J. Respir. Cell Mol. Biol.* 25, 699-706.

[199] Madan, T, Eggleton, P, Kishore, U, Strong, P, Aggrawal, SS, Sarma, PU, and Reid, KB. (1997). Binding of pulmonary surfactant proteins A and D to Aspergillus fumigatus conidia enhances phagocytosis and killing by human neutrophils and alveolar macrophages. *Infect. Immun.* 65, 3171-9.

[200] Mariencheck, WI, Savov, J, Dong, Q, Tino, MJ, and Wright, JR. (1999). Surfactant protein A enhances alveolar macrophage phagocytosis of a live, mucoid strain of P. aeruginosa. *Am. J. Physiol.* 277, L777-86.

[201] Ofek, I, Mesika, A, Kalina, M, Keisari, Y, Podschun, R, Sahly, H, Chang, D, McGregor, D, and Crouch, E. (2001). Surfactant protein D enhances phagocytosis and killing of unencapsulated phase variants of Klebsiella pneumoniae. *Infect. Immun.* 69, 24-33.

[202] Pasula, R, Downing, JF, Wright, JR, Kachel, DL, Davis, TE,Jr, and Martin, WJ,2nd. (1997). Surfactant protein A (SP-A) mediates attachment of Mycobacterium tuberculosis to murine alveolar macrophages. *Am. J. Respir. Cell Mol. Biol.* 17, 209-17.

[203] Yong, SJ, Vuk-Pavlovic, Z, Standing, JE, Crouch, EC, and Limper, AH. (2003). Surfactant protein D-mediated aggregation of Pneumocystis carinii impairs phagocytosis by alveolar macrophages. *Infect. Immun.* 71, 1662-71.

[204] Restrepo, CI, Dong, Q, Savov, J, Mariencheck, WI, and Wright, JR. (1999). Surfactant protein D stimulates phagocytosis of Pseudomonas aeruginosa by alveolar macrophages. *Am. J. Respir. Cell Mol. Biol.* 21, 576-85.

[205] Szalai, AJ. (2002). The biological functions of C-reactive protein. *Vascul Pharmacol.* 39, 105-7.

[206] Deban, L, Bottazzi, B, Garlanda, C, de la Torre, YM, and Mantovani, A. (2009). Pentraxins: multifunctional proteins at the interface of innate immunity and inflammation. *Biofactors* 35, 138-45.

[207] Sugimoto, R, Yae, Y, Akaiwa, M, Kitajima, S, Shibata, Y, Sato, H, Hirata, J, Okochi, K, Izuhara, K, and Hamasaki, N. (1998). Cloning and characterization of the Hakata antigen, a member of the ficolin/opsonin p35 lectin family. *J. Biol. Chem.* 273, 20721-7.

[208] Matsushita, M, Endo, Y, Taira, S, Sato, Y, Fujita, T, Ichikawa, N, Nakata, M, and Mizuochi, T. (1996). A novel human serum lectin with collagen- and fibrinogen-like domains that functions as an opsonin. *J. Biol. Chem.* 271, 2448-54.

[209] Lu, J, Marnell, LL, Marjon, KD, Mold, C, Du Clos, TW, and Sun, PD. (2008). Structural recognition and functional activation of FcgammaR by innate pentraxins. *Nature* 456, 989-92.

[210] Ogden, CA, deCathelineau, A, Hoffmann, PR, Bratton, D, Ghebrehiwet, B, Fadok, VA, and Henson, PM. (2001). C1q and mannose binding lectin engagement of cell surface calreticulin and CD91 initiates macropinocytosis and uptake of apoptotic cells. *J. Exp. Med.* 194, 781-95.

[211] Ohya, M, Nishitani, C, Sano, H, Yamada, C, Mitsuzawa, H, Shimizu, T, Saito, T, Smith, K, Crouch, E, and Kuroki, Y. (2006). Human pulmonary surfactant protein D binds the extracellular domains of Toll-like receptors 2 and 4 through the carbohydrate recognition domain by a mechanism different from its binding to phosphatidylinositol and lipopolysaccharide. *Biochemistry* 45, 8657-64.

[212] Worthley, DL, Bardy, PG, and Mullighan, CG. (2005). Mannose-binding lectin: biology and clinical implications. *Intern. Med. J.* 35, 548-55.

[213] Klotz, L, Hucke, S, Thimm, D, Classen, S, Gaarz, A, Schultze, J, Edenhofer, F, Kurts, C, Klockgether, T, Limmer, A, Knolle, P, and Burgdorf, S. (2009). Increased antigen cross-presentation but impaired cross-priming after activation of peroxisome proliferator-activated receptor gamma is mediated by up-regulation of B7H1. *J. Immunol.* 183, 129-36.

[214] Glasser, SW, Witt, TL, Senft, AP, Baatz, JE, Folger, D, Maxfield, MD, Akinbi, HT, Newton, DA, Prows, DR, and Korfhagen, TR. (2009). Surfactant protein C-deficient mice are susceptible to respiratory syncytial virus infection. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 297, L64-72.

[215] Ghazizadeh, S and Fleit, HB. (1994). Tyrosine phosphorylation provides an obligatory early signal for Fc gamma RII-mediated endocytosis in the monocytic cell line THP-1. *J. Immunol.* 152, 30-41.

[216] Sobota, A, Strzelecka-Kiliszek, A, Gladkowska, E, Yoshida, K, Mrozinska, K, and Kwiatkowska, K. (2005). Binding of IgG-opsonized particles to Fc gamma R is an active stage of phagocytosis that involves receptor clustering and phosphorylation. *J. Immunol.* 175, 4450-7.

[217] DerMardirossian, C and Bokoch, GM. (2005). GDIs: central regulatory molecules in Rho GTPase activation. *Trends Cell Biol.* 15, 356-63.

[218] Goley, ED and Welch, MD. (2006). The ARP2/3 complex: an actin nucleator comes of age. *Nat. Rev. Mol. Cell Biol.* 7, 713-26.

[219] Caron, E and Hall, A. (1998). Identification of two distinct mechanisms of phagocytosis controlled by different Rho GTPases. *Science* 282, 1717-21.

[220] Yamauchi, A, Kim, C, Li, S, Marchal, CC, Towe, J, Atkinson, SJ, and Dinauer, MC. (2004). Rac2-deficient murine macrophages have selective defects in superoxide production and phagocytosis of opsonized particles. *J. Immunol.* 173, 5971-9.

[221] Hoppe, AD and Swanson, JA. (2004). Cdc42, Rac1, and Rac2 display distinct patterns of activation during phagocytosis. *Mol. Biol. Cell* 15, 3509-19.

[222] Wear, MA, Schafer, DA, and Cooper, JA. (2000). Actin dynamics: assembly and disassembly of actin networks. *Curr. Biol.* 10, R891-5.

[223] Griffin, FM,Jr, Bianco, C, and Silverstein, SC. (1975). Characterization of the macrophage receptro for complement and demonstration of its functional independence from the receptor for the Fc portion of immunoglobulin G. *J. Exp. Med.* 141, 1269-77.

[224] Kaplan, G. (1977). Differences in the mode of phagocytosis with Fc and C3 receptors in macrophages. *Scand. J. Immunol.* 6, 797-807.

[225] Allen, LA and Aderem, A. (1996). Molecular definition of distinct cytoskeletal structures involved in complement- and Fc receptor-mediated phagocytosis in macrophages. *J. Exp. Med.* 184, 627-37.

[226] Bianco, C, Griffin, FM,Jr, and Silverstein, SC. (1975). Studies of the macrophage complement receptor. Alteration of receptor function upon macrophage activation. *J. Exp. Med.* 141, 1278-90.

[227] Caron, E, Self, AJ, and Hall, A. (2000). The GTPase Rap1 controls functional activation of macrophage integrin alphaMbeta2 by LPS and other inflammatory mediators. *Curr. Biol.* 10, 974-8.

[228] Wiedemann, A, Patel, JC, Lim, J, Tsun, A, van Kooyk, Y, and Caron, E. (2006). Two distinct cytoplasmic regions of the beta2 integrin chain regulate RhoA function during phagocytosis. *J. Cell Biol.* 172, 1069-79.

[229] Worgall, S, Bezdicek, P, Kim, MK, Park, JG, Singh, R, Christofidou-Solomidou, M, Prince, A, Kovesdi, I, Schreiber, AD, and Crystal, RG. (1999). Augmentation of pulmonary host defense against Pseudomonas by FcgammaRIIA cDNA transfer to the respiratory epithelium. *J. Clin. Invest.* 104, 409-18.

[230] Kang, BK and Schlesinger, LS. (1998). Characterization of mannose receptor-dependent phagocytosis mediated by Mycobacterium tuberculosis lipoarabinomannan. *Infect. Immun.* 66, 2769-77.

[231] Kang, PB, Azad, AK, Torrelles, JB, Kaufman, TM, Beharka, A, Tibesar, E, DesJardin, LE, and Schlesinger, LS. (2005). The human macrophage mannose receptor directs Mycobacterium tuberculosis lipoarabinomannan-mediated phagosome biogenesis. *J. Exp. Med.* 202, 987-99.

[232] Chieppa, M, Bianchi, G, Doni, A, Del Prete, A, Sironi, M, Laskarin, G, Monti, P, Piemonti, L, Biondi, A, Mantovani, A, Introna, M, and Allavena, P. (2003). Cross-linking of the mannose receptor on monocyte-derived dendritic cells activates an anti-inflammatory immunosuppressive program. *J. Immunol.* 171, 4552-60.

[233] Schulert, GS and Allen, LA. (2006). Differential infection of mononuclear phagocytes by Francisella tularensis: role of the macrophage mannose receptor. *J. Leukoc. Biol.* 80, 563-71.

[234] Kruskal, BA, Sastry, K, Warner, AB, Mathieu, CE, and Ezekowitz, RA. (1992). Phagocytic chimeric receptors require both transmembrane and cytoplasmic domains from the mannose receptor. *J. Exp. Med.* 176, 1673-80.

[235] Zhang, J, Zhu, J, Bu, X, Cushion, M, Kinane, TB, Avraham, H, and Koziel, H. (2005). Cdc42 and RhoB activation are required for mannose receptor-mediated phagocytosis by human alveolar macrophages. *Mol. Biol. Cell* 16, 824-34.

[236] Le Cabec, V, Emorine, LJ, Toesca, I, Cougoule, C, and Maridonneau-Parini, I. (2005). The human macrophage mannose receptor is not a professional phagocytic receptor. *J. Leukoc. Biol.* 77, 934-43.

[237] Zhou, T, Chen, Y, Hao, L, and Zhang, Y. (2006). DC-SIGN and immunoregulation. *Cell. Mol. Immunol.* 3, 279-83.

[238] Iyori, M, Ohtani, M, Hasebe, A, Totsuka, Y, and Shibata, K. (2008). A role of the Ca2+ binding site of DC-SIGN in the phagocytosis of E. coli. *Biochem. Biophys. Res. Commun.* 377, 367-72.

[239] Hodges, A, Sharrocks, K, Edelmann, M, Baban, D, Moris, A, Schwartz, O, Drakesmith, H, Davies, K, Kessler, B, McMichael, A, and Simmons, A. (2007). Activation of the lectin DC-SIGN induces an immature dendritic cell phenotype triggering Rho-GTPase activity required for HIV-1 replication. *Nat. Immunol.* 8, 569-77.

[240] Ariizumi, K, Shen, GL, Shikano, S, Xu, S, Ritter, R,3rd, Kumamoto, T, Edelbaum, D, Morita, A, Bergstresser, PR, and Takashima, A. (2000). Identification of a novel, dendritic cell-associated molecule, dectin-1, by subtractive cDNA cloning. *J. Biol. Chem.* 275, 20157-67.

[241] Brown, GD and Gordon, S. (2001). Immune recognition. A new receptor for beta-glucans. *Nature* 413, 36-7.

[242] Brown, GD and Gordon, S. (2003). Fungal beta-glucans and mammalian immunity. *Immunity* 19, 311-5.

[243] Herre, J, Willment, JA, Gordon, S, and Brown, GD. (2004). The role of Dectin-1 in antifungal immunity. *Crit. Rev. Immunol.* 24, 193-203.

[244] Mantegazza, AR, Barrio, MM, Moutel, S, Bover, L, Weck, M, Brossart, P, Teillaud, JL, and Mordoh, J. (2004). CD63 tetraspanin slows down cell migration and translocates to the endosomal-lysosomal-MIICs route after extracellular stimuli in human immature dendritic cells. *Blood* 104, 1183-90.

[245] Kong, L and Ge, BX. (2008). MyD88-independent activation of a novel actin-Cdc42/Rac pathway is required for Toll-like receptor-stimulated phagocytosis. *Cell Res.* 18, 745-55.

[246] Rogers, NC, Slack, EC, Edwards, AD, Nolte, MA, Schulz, O, Schweighoffer, E, Williams, DL, Gordon, S, Tybulewicz, VL, Brown, GD, and Reis e Sousa, C. (2005). Syk-dependent cytokine induction by Dectin-1 reveals a novel pattern recognition pathway for C type lectins. *Immunity* 22, 507-17.

[247] Bonifaz, LC, Bonnyay, DP, Charalambous, A, Darguste, DI, Fujii, S, Soares, H, Brimnes, MK, Moltedo, B, Moran, TM, and Steinman, RM. (2004). In vivo targeting of antigens to maturing dendritic cells via the DEC-205 receptor improves T cell vaccination. *J. Exp. Med.* 199, 815-24.

[248] Liu, Y, Gao, X, Masuda, E, Redecha, PB, Blank, MC, and Pricop, L. (2006). Regulated expression of FcgammaR in human dendritic cells controls cross-presentation of antigen-antibody complexes. *J. Immunol.* 177, 8440-7.

[249] Graham, DB, Stephenson, LM, Lam, SK, Brim, K, Lee, HM, Bautista, J, Gilfillan, S, Akilesh, S, Fujikawa, K, and Swat, W. (2007). An ITAM-signaling pathway controls cross-presentation of particulate but not soluble antigens in dendritic cells. *J. Exp. Med.* 204, 2889-97.

[250] Su, SV, Hong, P, Baik, S, Negrete, OA, Gurney, KB, and Lee, B. (2004). DC-SIGN binds to HIV-1 glycoprotein 120 in a distinct but overlapping fashion compared with ICAM-2 and ICAM-3. *J. Biol. Chem.* 279, 19122-32.

[251] Malcherek, G, Mayr, L, Roda-Navarro, P, Rhodes, D, Miller, N, and Trowsdale, J. (2007). The B7 homolog butyrophilin BTN2A1 is a novel ligand for DC-SIGN. *J. Immunol.* 179, 3804-11.

[252] Naarding, MA, Dirac, AM, Ludwig, IS, Speijer, D, Lindquist, S, Vestman, EL, Stax, MJ, Geijtenbeek, TB, Pollakis, G, Hernell, O, and Paxton, WA. (2006). Bile salt-stimulated lipase from human milk binds DC-SIGN and inhibits human immunodeficiency virus type 1 transfer to CD4+ T cells. *Antimicrob. Agents Chemother.* 50, 3367-74.

[253] van Gisbergen, KP, Sanchez-Hernandez, M, Geijtenbeek, TB, and van Kooyk, Y. (2005). Neutrophils mediate immune modulation of dendritic cells through glycosylation-dependent interactions between Mac-1 and DC-SIGN. *J. Exp. Med.* 201, 1281-92.

[254] van Gisbergen, KP, Aarnoudse, CA, Meijer, GA, Geijtenbeek, TB, and van Kooyk, Y. (2005). Dendritic cells recognize tumor-specific glycosylation of carcinoembryonic antigen on colorectal cancer cells through dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin. *Cancer Res.* 65, 5935-44.

[255] Bogoevska, V, Horst, A, Klampe, B, Lucka, L, Wagener, C, and Nollau, P. (2006). CEACAM1, an adhesion molecule of human granulocytes, is fucosylated by fucosyltransferase IX and interacts with DC-SIGN of dendritic cells via Lewis x residues. *Glycobiology* 16, 197-209.

[256] Leteux, C, Chai, W, Loveless, RW, Yuen, CT, Uhlin-Hansen, L, Combarnous, Y, Jankovic, M, Maric, SC, Misulovin, Z, Nussenzweig, MC, and Feizi, T. (2000). The cysteine-rich domain of the macrophage mannose receptor is a multispecific lectin that recognizes chondroitin sulfates A and B and sulfated oligosaccharides of blood group Lewis(a) and Lewis(x) types in addition to the sulfated N-glycans of lutropin. *J. Exp. Med.* 191, 1117-26.

[257] Taylor, PR, Gordon, S, and Martinez-Pomares, L. (2005). The mannose receptor: linking homeostasis and immunity through sugar recognition. *Trends Immunol.* 26, 104-10.

[258] Lee, SJ, Evers, S, Roeder, D, Parlow, AF, Risteli, J, Risteli, L, Lee, YC, Feizi, T, Langen, H, and Nussenzweig, MC. (2002). Mannose receptor-mediated regulation of serum glycoprotein homeostasis. *Science* 295, 1898-901.

[259] Moris, A, Nobile, C, Buseyne, F, Porrot, F, Abastado, JP, and Schwartz, O. (2004). DC-SIGN promotes exogenous MHC-I-restricted HIV-1 antigen presentation. *Blood* 103, 2648-54.

[260] Blander, JM and Medzhitov, R. (2006). Toll-dependent selection of microbial antigens for presentation by dendritic cells. *Nature* 440, 808-12.

[261] Takeda, K, Kaisho, T, and Akira, S. (2003). Toll-like receptors. *Annu. Rev. Immunol.* 21, 335-76.

[262] Doyle, SE, O'Connell, RM, Miranda, GA, Vaidya, SA, Chow, EK, Liu, PT, Suzuki, S, Suzuki, N, Modlin, RL, Yeh, WC, Lane, TF, and Cheng, G. (2004). Toll-like receptors induce a phagocytic gene program through p38. *J. Exp. Med.* 199, 81-90.

[263] Blander, JM and Medzhitov, R. (2004). Regulation of phagosome maturation by signals from toll-like receptors. *Science* 304, 1014-8.

[264] Yates, RM and Russell, DG. (2005). Phagosome maturation proceeds independently of stimulation of toll-like receptors 2 and 4. *Immunity* 23, 409-17.

[265] Tricker, E and Cheng, G. (2008). With a little help from my friends: modulation of phagocytosis through TLR activation. *Cell Res.* 18, 711-2.

[266] West, MA, Wallin, RP, Matthews, SP, Svensson, HG, Zaru, R, Ljunggren, HG, Prescott, AR, and Watts, C. (2004). Enhanced dendritic cell antigen capture via toll-like receptor-induced actin remodeling. *Science* 305, 1153-7.

[267] Vieira, OV, Botelho, RJ, and Grinstein, S. (2002). Phagosome maturation: aging gracefully. *Biochem. J.* 366, 689-704.

[268] Janssen, E, Tabeta, K, Barnes, MJ, Rutschmann, S, McBride, S, Bahjat, KS, Schoenberger, SP, Theofilopoulos, AN, Beutler, B, and Hoebe, K. (2006). Efficient T cell activation via a Toll-Interleukin 1 Receptor-independent pathway. *Immunity* 24, 787-99.

[269] Trombetta, ES and Mellman, I. (2005). Cell biology of antigen processing in vitro and in vivo. *Annu. Rev. Immunol.* 23, 975-1028.

[270] Blander, JM and Medzhitov, R. (2006). On regulation of phagosome maturation and antigen presentation. *Nat. Immunol.* 7, 1029-35.

[271] Cavalli, V, Vilbois, F, Corti, M, Marcote, MJ, Tamura, K, Karin, M, Arkinstall, S, and Gruenberg, J. (2001). The stress-induced MAP kinase p38 regulates endocytic trafficking via the GDI:Rab5 complex. *Mol. Cell* 7, 421-32.

[272] Sanjuan, MA, Dillon, CP, Tait, SW, Moshiach, S, Dorsey, F, Connell, S, Komatsu, M, Tanaka, K, Cleveland, JL, Withoff, S, and Green, DR. (2007). Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. *Nature* 450, 1253-7.

[273] Jutras, I, Houde, M, Currier, N, Boulais, J, Duclos, S, LaBoissiere, S, Bonneil, E, Kearney, P, Thibault, P, Paramithiotis, E, Hugo, P, and Desjardins, M. (2008). Modulation of the phagosome proteome by interferon-gamma. *Mol. Cell. Proteomics* 7, 697-715.

[274] Schroder, K, Hertzog, PJ, Ravasi, T, and Hume, DA. (2004). Interferon-gamma: an overview of signals, mechanisms and functions. *J. Leukoc. Biol.* 75, 163-89.

[275] Landmann, S, Muhlethaler-Mottet, A, Bernasconi, L, Suter, T, Waldburger, JM, Masternak, K, Arrighi, JF, Hauser, C, Fontana, A, and Reith, W. (2001). Maturation of dendritic cells is accompanied by rapid transcriptional silencing of class II transactivator (CIITA) expression. *J. Exp. Med.* 194, 379-91.

[276] Yao, Y, Xu, Q, Kwon, MJ, Matta, R, Liu, Y, Hong, SC, and Chang, CH. (2006). ERK and p38 MAPK signaling pathways negatively regulate CIITA gene expression in dendritic cells and macrophages. *J. Immunol.* 177, 70-6.

[277] Pierre, P and Mellman, I. (1998). Developmental regulation of invariant chain proteolysis controls MHC class II trafficking in mouse dendritic cells. *Cell* 93, 1135-45.

[278] Chow, A, Toomre, D, Garrett, W, and Mellman, I. (2002). Dendritic cell maturation triggers retrograde MHC class II transport from lysosomes to the plasma membrane. *Nature* 418, 988-94.

[279] Shin, JS, Ebersold, M, Pypaert, M, Delamarre, L, Hartley, A, and Mellman, I. (2006). Surface expression of MHC class II in dendritic cells is controlled by regulated ubiquitination. *Nature* 444, 115-8.

[280] Robinson, DS and O'Garra, A. (2002). Further checkpoints in Th1 development. *Immunity* 16, 755-8.

[281] Garcon, N, Heppner, DG, and Cohen, J. (2003). Development of RTS,S/AS02: a purified subunit-based malaria vaccine candidate formulated with a novel adjuvant. *Expert Rev Vaccines* 2, 231-8.

[282] Smith-Garvin, JE, Koretzky, GA, and Jordan, MS. (2009). T cell activation. *Annu. Rev. Immunol.* 27, 591-619.

[283] Mostbock, S, Vidal, S, Schlom, J, and Sabzevari, H. (2007). Enhanced levels of costimulation lead to reduced effector/memory CD8+ T cell functionality. *J. Immunol.* 179, 3524-34.

[284] Gillet-Hladky, S, Duperrier, K, Picandet, S, Mathias, V, de Carvalho, MC, Bernaud, J, Masseau, D, Bienvenu, J, and Rigal, D. (2008). Induction of high expression of CCR7 and high production of IL-12 in human monocyte-derived dendritic cells by a new bacterial component: LCOS 1013. *Int. Immunopharmacol.* 8, 1151-63.

[285] Takeuchi, O, Kaufmann, A, Grote, K, Kawai, T, Hoshino, K, Morr, M, Muhlradt, PF, and Akira, S. (2000). Cutting edge: preferentially the R-stereoisomer of the mycoplasmal lipopeptide macrophage-activating lipopeptide-2 activates immune cells through a toll-like receptor 2- and MyD88-dependent signaling pathway. *J. Immunol.* 164, 554-7.

[286] Iwasaki, A and Medzhitov, R. (2004). Toll-like receptor control of the adaptive immune responses. *Nat. Immunol.* 5, 987-95.

[287] Kagan, JC, Su, T, Horng, T, Chow, A, Akira, S, and Medzhitov, R. (2008). TRAM couples endocytosis of Toll-like receptor 4 to the induction of interferon-beta. *Nat. Immunol.* 9, 361-8.

[288] Reis e Sousa, C. (2006). Dendritic cells in a mature age. *Nat. Rev. Immunol.* 6, 476-83.

[289] Re, F and Strominger, JL. (2001). Toll-like receptor 2 (TLR2) and TLR4 differentially activate human dendritic cells. *J. Biol. Chem.* 276, 37692-9.

[290] Carmody, RJ, Ruan, Q, Liou, HC, and Chen, YH. (2007). Essential roles of c-Rel in TLR-induced IL-23 p19 gene expression in dendritic cells. *J. Immunol.* 178, 186-91.

[291] LeibundGut-Landmann, S, Gross, O, Robinson, MJ, Osorio, F, Slack, EC, Tsoni, SV, Schweighoffer, E, Tybulewicz, V, Brown, GD, Ruland, J, and Reis e Sousa, C. (2007). Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. *Nat. Immunol.* 8, 630-8.

[292] Osorio, F, LeibundGut-Landmann, S, Lochner, M, Lahl, K, Sparwasser, T, Eberl, G, and Reis e Sousa, C. (2008). DC activated via dectin-1 convert Treg into IL-17 producers. *Eur. J. Immunol.* 38, 3274-81.

[293] Gringhuis, SI, den Dunnen, J, Litjens, M, van Het Hof, B, van Kooyk, Y, and Geijtenbeek, TB. (2007). C-type lectin DC-SIGN modulates Toll-like receptor signaling via Raf-1 kinase-dependent acetylation of transcription factor NF-kappaB. *Immunity* 26, 605-16.

[294] Nedelec, J, Boucraut, J, Garnier, JM, Bernard, D, and Rougon, G. (1990). Evidence for autoimmune antibodies directed against embryonic neural cell adhesion molecules (N-CAM) in patients with group B meningitis. *J. Neuroimmunol.* 29, 49-56.

[295] Comstock, LE and Kasper, DL. (2006). Bacterial glycans: key mediators of diverse host immune responses. *Cell* 126, 847-50.

[296] Mosier, DE, Zaldivar, NM, Goldings, E, Mond, J, Scher, I, and Paul, WE. (1977). Formation of antibody in the newborn mouse: study of T-cell-independent antibody response. *J. Infect. Dis.* 136 Suppl, S14-9.

[297] Haneberg, B, Dalseg, R, Wedege, E, Hoiby, EA, Haugen, IL, Oftung, F, Andersen, SR, Naess, LM, Aase, A, Michaelsen, TE, and Holst, J. (1998). Intranasal administration of a meningococcal outer membrane vesicle vaccine induces persistent local mucosal antibodies and serum antibodies with strong bactericidal activity in humans. *Infect. Immun.* 66, 1334-41.

[298] Bondada, S, Wu, H, Robertson, DA, and Chelvarajan, RL. (2000). Accessory cell defect in unresponsiveness of neonates and aged to polysaccharide vaccines. *Vaccine* 19, 557-65.

[299] Wessels, MR, Paoletti, LC, Rodewald, AK, Michon, F, DiFabio, J, Jennings, HJ, and Kasper, DL. (1993). Stimulation of protective antibodies against type Ia and Ib group B streptococci by a type Ia polysaccharide-tetanus toxoid conjugate vaccine. *Infect. Immun.* 61, 4760-6.

[300] Schneerson, R, Barrera, O, Sutton, A, and Robbins, JB. (1980). Preparation, characterization, and immunogenicity of Haemophilus influenzae type b polysaccharide-protein conjugates. *J. Exp. Med.* 152, 361-76.

[301] Guttormsen, HK, Sharpe, AH, Chandraker, AK, Brigtsen, AK, Sayegh, MH, and Kasper, DL. (1999). Cognate stimulatory B-cell-T-cell interactions are critical for T-cell help recruited by glycoconjugate vaccines. *Infect. Immun.* 67, 6375-84.

[302] Nudelman, Y and Tunkel, AR. (2009). Bacterial meningitis: epidemiology, pathogenesis and management update. *Drugs* 69, 2577-96.

[303] Tzianabos, AO, Chandraker, A, Kalka-Moll, W, Stingele, F, Dong, VM, Finberg, RW, Peach, R, and Sayegh, MH. (2000). Bacterial pathogens induce abscess formation by CD4(+) T-cell activation via the CD28-B7-2 costimulatory pathway. *Infect. Immun.* 68, 6650-5.

[304] Tzianabos, A, Wang, JY, and Kasper, DL. (2003). Biological chemistry of immunomodulation by zwitterionic polysaccharides. *Carbohydr. Res.* 338, 2531-8.

[305] Stephen, TL, Niemeyer, M, Tzianabos, AO, Kroenke, M, Kasper, DL, and Kalka-Moll, WM. (2005). Effect of B7-2 and CD40 signals from activated antigen-presenting cells on the ability of zwitterionic polysaccharides to induce T-Cell stimulation. *Infect. Immun.* 73, 2184-9.

[306] Polk, BF and Kasper, DL. (1977). Bacteroides fragilis subspecies in clinical isolates. *Ann. Intern. Med.* 86, 569-71.

[307] Weinstein, WM, Onderdonk, AB, Bartlett, JG, and Gorbach, SL. (1974). Experimental intra-abdominal abscesses in rats: development of an experimental model. *Infect. Immun.* 10, 1250-5.

[308] Kasper, DL, Onderdonk, AB, Crabb, J, and Bartlett, JG. (1979). Protective efficacy of immunization with capsular antigen against experimental infection with Bacteroides fragilis. *J. Infect. Dis.* 140, 724-31.

[309] Tzianabos, AO, Kasper, DL, Cisneros, RL, Smith, RS, and Onderdonk, AB. (1995). Polysaccharide-mediated protection against abscess formation in experimental intra-abdominal sepsis. *J. Clin. Invest.* 96, 2727-31.

[310] Tzianabos, AO, Wang, JY, and Lee, JC. (2001). Structural rationale for the modulation of abscess formation by Staphylococcus aureus capsular polysaccharides. *Proc. Natl. Acad. Sci. U. S. A.* 98, 9365-70.

[311] Tzianabos, AO, Finberg, RW, Wang, Y, Chan, M, Onderdonk, AB, Jennings, HJ, and Kasper, DL. (2000). T cells activated by zwitterionic molecules prevent abscesses induced by pathogenic bacteria. *J. Biol. Chem.* 275, 6733-40.

[312] Kalka-Moll, WM, Tzianabos, AO, Bryant, PW, Niemeyer, M, Ploegh, HL, and Kasper, DL. (2002). Zwitterionic polysaccharides stimulate T cells by MHC class II-dependent interactions. *J. Immunol.* 169, 6149-53.

[313] Avci, FY and Kasper, DL. (2009). How Bacterial Carbohydrates Influence the Adaptive Immune System. *Annu. Rev. Immunol.* .

[314] Tzianabos, AO, Onderdonk, AB, Rosner, B, Cisneros, RL, and Kasper, DL. (1993). Structural features of polysaccharides that induce intra-abdominal abscesses. *Science* 262, 416-9.

[315] Stingele, F, Corthesy, B, Kusy, N, Porcelli, SA, Kasper, DL, and Tzianabos, AO. (2004). Zwitterionic polysaccharides stimulate T cells with no preferential V beta usage and promote anergy, resulting in protection against experimental abscess formation. *J. Immunol.* 172, 1483-90.

[316] Brubaker, JO, Li, Q, Tzianabos, AO, Kasper, DL, and Finberg, RW. (1999). Mitogenic activity of purified capsular polysaccharide A from Bacteroides fragilis: differential stimulatory effect on mouse and rat lymphocytes in vitro. *J. Immunol.* 162, 2235-42.

[317] Choi, YH, Roehrl, MH, Kasper, DL, and Wang, JY. (2002). A unique structural pattern shared by T-cell-activating and abscess-regulating zwitterionic polysaccharides. *Biochemistry* 41, 15144-51.

[318] Moreau, M, Richards, JC, Fournier, JM, Byrd, RA, Karakawa, WW, and Vann, WF. (1990). Structure of the type 5 capsular polysaccharide of Staphylococcus aureus. *Carbohydr. Res.* 201, 285-97.

[319] Wang, Y, Kalka-Moll, WM, Roehrl, MH, and Kasper, DL. (2000). Structural basis of the abscess-modulating polysaccharide A2 from Bacteroides fragilis. *Proc. Natl. Acad. Sci. U. S. A.* 97, 13478-83.

[320] Deehan, MR, Frame, MJ, Parkhouse, RM, Seatter, SD, Reid, SD, Harnett, MM, and Harnett, W. (1998). A phosphorylcholine-containing filarial nematode-secreted product disrupts B lymphocyte activation by targeting key proliferative signaling pathways. *J. Immunol.* 160, 2692-9.

[321] Harnett, MM, Deehan, MR, Williams, DM, and Harnett, W. (1998). Induction of signalling anergy via the T-cell receptor in cultured Jurkat T cells by pre-exposure to a filarial nematode secreted product. *Parasite Immunol.* 20, 551-63.

[322] Lal, RB, Kumaraswami, V, Steel, C, and Nutman, TB. (1990). Phosphocholine-containing antigens of Brugia malayi nonspecifically suppress lymphocyte function. *Am. J. Trop. Med. Hyg.* 42, 56-64.

[323] Lochnit, G, Dennis, RD, Ulmer, AJ, and Geyer, R. (1998). Structural elucidation and monokine-inducing activity of two biologically active zwitterionic glycosphingolipids derived from the porcine parasitic nematode Ascaris suum. *J. Biol. Chem.* 273, 466-74.

[324] Cobb, BA and Kasper, DL. (2008). Characteristics of carbohydrate antigen binding to the presentation protein HLA-DR. *Glycobiology* 18, 707-18.

[325] Kreisman, LS, Friedman, JH, Neaga, A, and Cobb, BA. (2007). Structure and function relations with a T-cell-activating polysaccharide antigen using circular dichroism. *Glycobiology* 17, 46-55.

[326] Kalka-Moll, WM, Tzianabos, AO, Wang, Y, Carey, VJ, Finberg, RW, Onderdonk, AB, and Kasper, DL. (2000). Effect of molecular size on the ability of zwitterionic polysaccharides to stimulate cellular immunity. *J. Immunol.* 164, 719-24.

[327] Jardetzky, TS, Brown, JH, Gorga, JC, Stern, LJ, Urban, RG, Chi, YI, Stauffacher, C, Strominger, JL, and Wiley, DC. (1994). Three-dimensional structure of a human class II histocompatibility molecule complexed with superantigen. *Nature* 368, 711-8.

[328] Stephen, TL, Fabri, M, Groneck, L, Rohn, TA, Hafke, H, Robinson, N, Rietdorf, J, Schrama, D, Becker, JC, Plum, G, Kronke, M, Kropshofer, H, and Kalka-Moll, WM. (2007). Transport of Streptococcus pneumoniae capsular polysaccharide in MHC Class II tubules. *PLoS Pathog.* 3, e32.

[329] Cobb, BA, Wang, Q, Tzianabos, AO, and Kasper, DL. (2004). Polysaccharide processing and presentation by the MHCII pathway. *Cell* 117, 677-87.

[330] Savina, A, Jancic, C, Hugues, S, Guermonprez, P, Vargas, P, Moura, IC, Lennon-Dumenil, AM, Seabra, MC, Raposo, G, and Amigorena, S. (2006). NOX2 controls phagosomal pH to regulate antigen processing during crosspresentation by dendritic cells. *Cell* 126, 205-18.

[331] Duan, J, Avci, FY, and Kasper, DL. (2008). Microbial carbohydrate depolymerization by antigen-presenting cells: deamination prior to presentation by the MHCII pathway. *Proc. Natl. Acad. Sci. U. S. A.* 105, 5183-8.

[332] Groneck, L, Schrama, D, Fabri, M, Stephen, TL, Harms, F, Meemboor, S, Hafke, H, Bessler, M, Becker, JC, and Kalka-Moll, WM. (2009). Oligoclonal CD4+ T cells promote host memory immune responses to Zwitterionic polysaccharide of Streptococcus pneumoniae. *Infect. Immun.* 77, 3705-12.

[333] Gallorini, S, Berti, F, Parente, P, Baronio, R, Aprea, S, D'Oro, U, Pizza, M, Telford, JL, and Wack, A. (2007). Introduction of zwitterionic motifs into bacterial polysaccharides generates TLR2 agonists able to activate APCs. *J. Immunol.* 179, 8208-15.

[334] Acuto, O and Michel, F. (2003). CD28-mediated co-stimulation: a quantitative support for TCR signalling. *Nat. Rev. Immunol.* 3, 939-51.

[335] Dall'Era, M and Davis, J. (2004). CTLA4Ig: a novel inhibitor of costimulation. *Lupus* 13, 372-6.

[336] Ruiz-Perez, B, Chung, DR, Sharpe, AH, Yagita, H, Kalka-Moll, WM, Sayegh, MH, Kasper, DL, and Tzianabos, AO. (2005). Modulation of surgical fibrosis by microbial zwitterionic polysaccharides. *Proc. Natl. Acad. Sci. U. S. A.* 102, 16753-8.

[337] Gibson, FC,3rd, Tzianabos, AO, and Onderdonk, AB. (1996). The capsular polysaccharide complex of Bacteroides fragilis induces cytokine production from human and murine phagocytic cells. *Infect. Immun.* 64, 1065-9.

[338] Meemboor, S, Mertens, J, Flenner, E, Groneck, L, Zingarelli, A, Gamstatter, T, Bessler, M, Seeger, JM, Kashkar, H, Odenthal, M, and Kalka-Moll, WM. (2009). Interleukin-6 is essential for zwitterionic polysaccharide-mediated abscess formation. *Innate Immun.* .

[339] Hirano, T. (1998). Interleukin 6 and its receptor: ten years later. *Int. Rev. Immunol.* 16, 249-84.

[340] Mitsuyama, K, Sata, M, and Rose-John, S. (2006). Interleukin-6 trans-signaling in inflammatory bowel disease. *Cytokine Growth Factor Rev.* 17, 451-61.

[341] Tzianabos, AO, Russell, PR, Onderdonk, AB, Gibson, FC,3rd, Cywes, C, Chan, M, Finberg, RW, and Kasper, DL. (1999). IL-2 mediates protection against abscess formation in an experimental model of sepsis. *J. Immunol.* 163, 893-7.

[342] Mazmanian, SK, Round, JL, and Kasper, DL. (2008). A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature* 453, 620-5.

[343] Mazmanian, SK, Liu, CH, Tzianabos, AO, and Kasper, DL. (2005). An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* 122, 107-18.

[344] Mertens, J, Fabri, M, Zingarelli, A, Kubacki, T, Meemboor, S, Groneck, L, Seeger, J, Bessler, M, Hafke, H, Odenthal, M, Bieler, JG, Kalka, C, Schneck, JP, Kashkar, H, and Kalka-Moll, WM. (2009). Streptococcus pneumoniae serotype 1 capsular polysaccharide induces CD8CD28 regulatory T

lymphocytes by TCR crosslinking. *PLoS Pathog.* 5, e1000596.

[345] Wang, Q, McLoughlin, RM, Cobb, BA, Charrel-Dennis, M, Zaleski, KJ, Golenbock, D, Tzianabos, AO, and Kasper, DL. (2006). A bacterial carbohydrate links innate and adaptive responses through Toll-like receptor 2. *J. Exp. Med.* 203, 2853-63.

[346] Mempel, M, Voelcker, V, Kollisch, G, Plank, C, Rad, R, Gerhard, M, Schnopp, C, Fraunberger, P, Walli, AK, Ring, J, Abeck, D, and Ollert, M. (2003). Toll-like receptor expression in human keratinocytes: nuclear factor kappaB controlled gene activation by Staphylococcus aureus is toll-like receptor 2 but not toll-like receptor 4 or platelet activating factor receptor dependent. *J. Invest. Dermatol.* 121, 1389-96.

[347] Bougneres, L, Helft, J, Tiwari, S, Vargas, P, Chang, BH, Chan, L, Campisi, L, Lauvau, G, Hugues, S, Kumar, P, Kamphorst, AO, Dumenil, AM, Nussenzweig, M, MacMicking, JD, Amigorena, S, and Guermonprez, P. (2009). A role for lipid bodies in the cross-presentation of phagocytosed antigens by MHC class I in dendritic cells. *Immunity* 31, 232-44.

[348] Hawiger, D, Inaba, K, Dorsett, Y, Guo, M, Mahnke, K, Rivera, M, Ravetch, JV, Steinman, RM, and Nussenzweig, MC. (2001). Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo. *J. Exp. Med.* 194, 769-79.

[349] Macagno, A, Molteni, M, Rinaldi, A, Bertoni, F, Lanzavecchia, A, Rossetti, C, and Sallusto, F. (2006). A cyanobacterial LPS antagonist prevents endotoxin shock and blocks sustained TLR4 stimulation required for cytokine expression. *J. Exp. Med.* 203, 1481-92.

[350] Napolitani, G, Rinaldi, A, Bertoni, F, Sallusto, F, and Lanzavecchia, A. (2005). Selected Toll-like receptor agonist combinations synergistically trigger a T helper type 1-polarizing program in dendritic cells. *Nat. Immunol.* 6, 769-76.