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Toll-like receptor 9 and Systemic Lupus Erythromatosus: novel insights

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ABSTRACT

Toll-like receptor 9 (TLR9) is a pattern recognition receptor (PRR) which senses danger by recognizing DNA that is taken up into endosomal compartments of immune cells, most importantly of plasmacytoid dendritic cells. Activation of these cells by DNA leads to the generation of a strong, interferon-mediated Th1 response. However, also regulatory functions for TLR9 activation have been reported.

Novel evidence suggests that the distinction between self- and foreign DNA by TLR9 is much more limited than previously thought, and depends on the localization of the antigen rather than sequence motifs. The endolysosomal localization is also required to prime TLR9 for activation by pH dependent proteolytic processing of its antigen binding domain. Therefore the localization of TLR9 is carefully controlled by a transport mechanism which contains the newly identified protein UNC93B.

The lack of specificity in DNA sensing emphasizes the risk TLR9 poses in respect to developing autoimmune diseases. Indeed, TLR9 polymorphisms have been correlated with multiple autoimmune diseases. The best studied TLR9-related autoimmune disease thus far is Systemic Lupus Erythromatosus (SLE). In SLE, TLR9 seems to have activating, but also suppressive roles. HMGB1, which was recently identified as a stimulator of CpG-mediated TLR9 activation, may also play a role in SLE.

Stable synthetic oligodeoxiribonucleotides with different activating and suppressing capacities provide a spectrum of potential drugs and adjuvants for future therapeutic strategies.

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INTRODUCTION

Our body's defense mechanisms are developed to recognize invaders and damaged cells and to remove them in a timely manner while keeping the impact on the healthy tissue as limited as possible. The adaptive immune system is armed to tackle invaders in a highly specific manner using tailor-made recognition molecules and various approaches depending on the identity of the target. Key to the adaptive immune system is the development of immunological memory, which is a representation of the strategies and molecules one developed to tackle pathogens encountered during one's lifetime. Although effective, the development of an adaptive immune response is slow and thus does not suffice to provide the first line of defense.

The innate immune system is a collective of proteins and cell types that, unlike the adaptive immune system, is already fully developed upon birth. Cells and proteins of the innate immune system respond within minutes after invasion and serve various tasks. These include both search-and-destroy tasks and intelligence tasks: gathering of information to activate and direct the development of the adaptive immune response.

Immune cells need to recognize invaders, invaded cells, and tumor cells to be able to take them out. Cells of the adaptive immune system require presentation of foreign peptide in MHC-peptide complexes for activation and for cell-mediated cytotoxic killing of infected cells. Also, they require signals from infected cells, surrounding tissue, and/or activated innate immune cells as co-stimulatory signals by which they determine whether activation is required, and what type of response would be necessary. Recognition of danger by innate immune cells depends on molecules that do not occur in the healthy host or that are aberrantly localized. Such molecules and the proteins that recognize them are called (pathogen-associated) molecular patterns (PAMPs) and pattern recognition receptors (PRRs) respectively.

The role of DNA as a molecular pattern is an interesting one. After all, both pathogens and the host itself possess this molecule, which suggests that there is a risk involved in utilizing it as an immunological danger signal. Also the current biochemical technologies allow us to design and produce DNA of our liking. This has opened avenues for the use of DNA as an

immunostimulatory agent in vaccines and possibly for understanding and treatment of autoimmune diseases. In this thesis I discuss novel findings on the DNA-recognizing PRR Toll-like receptor 9 and its roles in the autoimmune disease Systemic Lupus Erythromatosus.

DNA AS A TRIGGER FOR IMMUNE ACTIVATION.

Immune activation by pathogen-specific molecules such as the bacterial protein flagellin and lipopolysaccharides is easy to accept. That nucleic acids can trigger immune responses however, is a paradox between pattern recognition and the distinction of self versus foreign. After all, both the host and the pathogen make use of nucleic acids to encode and translate their genomic information.

UNMETHYLATED CPG DNA: HALLMARK OF BACTERIAL DNA

Methylation of DNA is a process that plays important roles in animals, plants, fungi, and bacteria alike. Its functions in vertebrates include gene regulation, cell differentiation, and DNA duplication. In bacteria, methylation of target sites for host endonucleases serves as protection from degeneration of host DNA, while the DNA of invading pathogens remains susceptible to destruction.

Dnmt1 is the methylase that is responsible for virtually all DNA methylation in animals. This highly conserved methylase, known in plants as Met1, methylates the cytosine of a dinucleotide CG (also known as CpG: a cytosine bound to a guanine via a single phosphate) on the 5th carbon atom, with a preference for hemi-methylated DNA. Thus it is capable of propagating methylation patterns in daughter cells to assure maintainance of cell differentiation throughout generations of cells. Interestingly, CpG DNA is statistically underrepresented by a three- to fourfold in vertebrates, whereas it is normally represented in bacterial DNA and large DNA viruses. Bacteria do not methylate their CpG motifs, possibly because of the high frequency of occurrence in their genomes.

Unmethylated CpG motifs were first proven to be immuno-stimulatory for B cells in 1995 by Krieg *et al*. [4] Three years earlier it was already shown that bacterial DNA could activate NK cells and inhibit tumor growth, while vertebrate DNA could not. [5] Interestingly, the exact bases surrounding the CpG motif were postulated as important for the immunogenicity of the CpG motif. In humans, GTCGTT is the most immunogenic CpG motif. In contrast, CCGG motifs are the least immunogenic and most common in humans. [6] TLR9 was identified in 2000 as the receptor for CpG-motif containing ssDNA. [7]

TLR9: A TOLL FOR DNA

Toll like receptors (TLRs) are a family of highly conserved transmembrane pattern recognition receptors that recognize components of pathogens in the extracellular and luminal milieus of the cell. The majority of the thus far thirteen identified mammalian TLRs perform this function on the cell surface, while a distinct selection samples vesicles of the endocytic pathway. Corresponding to their location, the first group focusses on bacterial and viral surface components such as lipo-polysaccharides and peptidoglycan. The latter group notably consists of the nucleic acid responsive TLRs 3, 7/8, and 9. TLR9 is the only TLR thus far found to recognize DNA. The protein is expressed in various cell types, but most notably in B cells and plasmacytoid dendritic cells.

LUMINAL DOMAIN

Considering the large variation in substrate-specificity ranging from hydrophilic to highly hydrophobic molecules and small synthetic compounds to macromolecules, the structures of the different TLRs are remarkably similar to each other. The human TLR9 monomer is a single-pass type 1 transmembrane protein of 1032 amino acids that roughly dissects into a bulky luminal substrate-recognition domain and a cytosolic signaling domain that connect via the transmembrane domain. An up-to-date review on TLR structures has been published this year in Immunity by MS Jin and JO Lee.[2]

Characteristic for the luminal domain of TLR9 is the predicted horseshoe shape of this domain, which is shared by all TLRs. This shape is caused by the presence of 27 leucine-rich repeat (LRR) motifs in the domain.[8] These are commonly found motifs in proteins, with a length of 20-30 amino acids that consist of a conserved "LxxLxLxxN" portion and a variable portion. The LRR motifs of TLRs belong to the "typical" subfamily of LRRs and have a length of 24 amino acids with a conserved "xLxxLxxLxLxxNxLxxLPxxFx" sequence. [2]

Crystallization of the luminal domain of TLRs is difficult and has not succeeded for TLR9 thus far. For TLRs 1, 2, 3, and 4 its structure has been solved by means of fusion constructs. Two types structures for this portion of the TLR molecule could be identified by X-ray crystallography: a "three-domain"-type of structure which seems exclusive for TLRs 1, 2, and 4, and a more regular horseshoe-shaped structure, which corresponds more closely to

the “typical” subfamily of LRRs. Based on sequence comparison with TLR 3, the luminal domain of TLR9 is most likely to correspond to the latter structure.

Recent data shows proteolytic processing of the luminal domain in an endosomal compartment is essential for activation of TLR9. The cystein proteases cathepsin-S and cathepsin-L are both capable of performing this function in vitro in absence of cells. [1] These proteases [9] and cathepsin K [10] were previously reported to be required for TLR9 activation in Ba/F3 cells and murine models for arthritis and experimental autoimmune encephalomyelitis, respectively. The proteolytic site of cathepsin cleavage was predicted to be located between amino acids 441 and 470 by mass spectrometry. (Fig 1.)[1] As the exact DNA-binding domain of TLR9 is still unknown, these results confine the search for this area to the more proximal part of the TLR9 luminal domain.

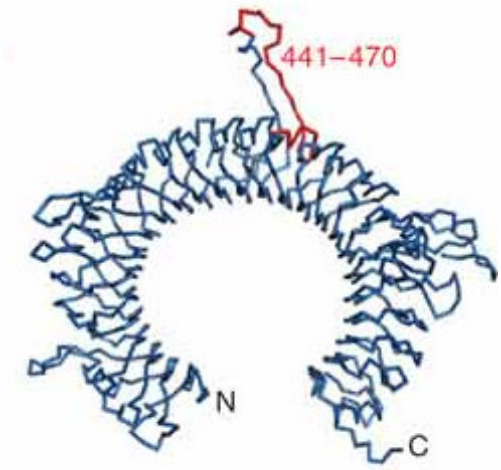

Figure 1: TLR9 cathepsin cleavage site

A model of the TLR9 luminal domain base don the structure of the TLR3 ectodomain. In red, the protruding loop that putatively contains the cathepsin cleavage site is highlighted. [1]

The localization of TLR9 was shown to be of importance for the distinction between self and non-self by Barton *et al.* in 2005, who showed that a chimeric TLR9 that was relocated to the cell surface, while equally responsive to synthetic ligands, failed to recognize viral DNA but was activated by self-DNA. For wildtype TLR9 exactly the opposite was observed.[11] Recently, Ewald *et al.* observed that, although both unprocessed and full length TLR9 can bind to ligand, only processed TLR9 co-immunoprecipitates with MyD88. [12] Thus it seems that only the processed variant of TLR9 is capable of signaling.

LOCALIZATION

The transmembrane domain of TLR9 is not simply an anchor for the protein through the membrane. It possibly plays a role in the regulation of TLR9 trafficking to vesicles of the endocytic pathway, where this protein is active. The currently most supported model of

TLR9 trafficking dictates that under steady-state conditions TLR9 resides in the endoplasmic reticulum. Upon activation by CpG-DNA, TLR9 is rapidly recruited to endolysosomes. A key player in transporting TLR9 to endolysosomes is a protein called UNC93B. This protein was discovered in 3d-mice,[13] which are homozygous for a point-mutation in one of the 12 transmembrane domains encoded in the *UNC93B1* gene,[14] and as a result are more susceptible to *L. monocytogenes*, *S. Aureus*, and MCMV infection. [13]

Work by the Ploegh lab revealed that this mutation in UNC93B retains the TLRs 3, 7 and 9 to the endoplasmic reticulum. Wildtype UNC93B interacts directly with TLRs 3, 7, 9, and 13, but this interaction does not occur with 3d-mutant UNC93B.[14] In the presence of wildtype UNC93B these TLRs are retained in the endoplasmic reticulum, but if the plasmamembrane sorting tag Ist-2p is attached to UNC93B, TLR3, 7, and 9 are actively transported to the plasmamembrane. Ist-2p modified mutant UNC93B however, failed to translocate TLRs to the plasmamembrane. [15] These results prove that UNC93B is a major player in intracellular endosomal TLR-trafficking. The specific interaction requirements of TLRs with UNC93B are unknown. The transmembrane region of TLRs have been reported to possess regulating activity, [11, 16], but was also reported not to be required for the trafficking of TLR9.[17] Since the 3d-mutation which abolishes interaction with TLRs is located in a transmembrane domain of UNC93B, a possible role for the transmembrane domain of TLR9 plays in its trafficking to endolysosomes cannot be excluded.

It has long been thought that TLR9 completely bypasses the Golgi-complex. A recent publication challenges this observation. Trafficking of TLR9 through the Golgi was reported to be essential for TLR9 capability to signal. [18] Also, Ewald et al. reported that, although the unprocessed product of TLR9 is susceptible to EndoH treatment, the processed variant of TLR is resistant to EndoH treatment, which indicates that this product specifically has traveled through the Golgi. [12] It is unlikely that UNC93B is involved in the transportation from endolysosomes to the Golgi-complex since UNC93B glycosylation was reported to be susceptible to EndoH treatment.[14]

The acidic conditions in the (late) endosome were shown to be important for the response to CpG-DNA, since blocking the acidification by adding the strong base chloroquine or by

blocking vesicle-specific H^+-pumps using bafilomycin A abolished the response to the synthetic CpG-DNA analog CpG-ODN. [19] DNA is an acidic molecule and pH could affect the charge and by that means the binding capacity of DNA. However, this is not likely the method by which pH abolishes CpG-DNA recognition by TLR9, since surface-expressed chimeric TLR9 constructs were shown to respond to CpG-DNA under physiological pH conditions. [11]

LIGAND SPECIFICITY

The initial experiments to unravel TLR9 specificity performed by Krieg et al. involved CpG-oligodeoxiribonucleotides (ODNs) that were stabilized with a phospho-thioate (PS) bond rather than the natural phospho-diester (PD) bond to prevent degradation. Using these synthetic CpG-ODNs, they showed that a species-specific consensus sequence of at least four nucleotides would be required to activate TLR9. [4] These results were challenged by the observation that TLR9 is capable of recognizing self-DNA [20] and non-CpG PD-DNA. [21] Further publications showing that poly-G-protected PD-ODNs that lack a CpG motive can bind to and activate TLR9 [22] conclusively showed that TLR9 has a much wider range of specificity than CpG-motifs and that its localization in endosomal compartments may thus have a protective effect preventing self-activation.

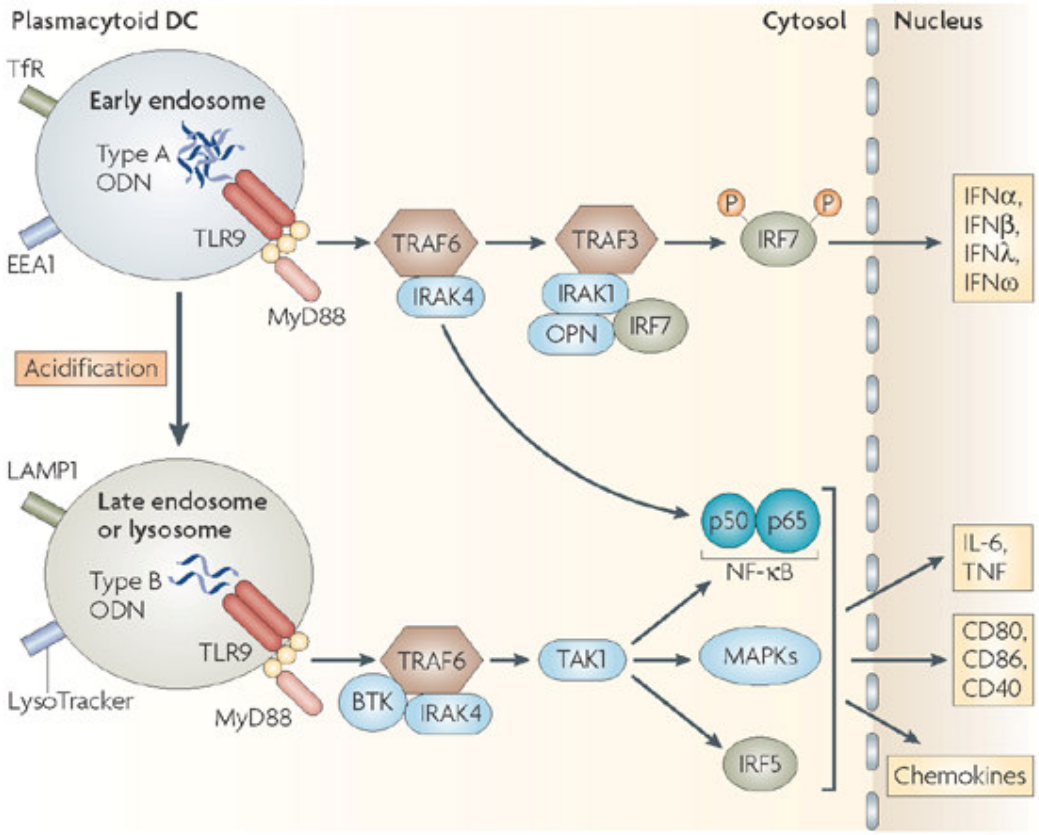

Figure 2. Differential signaling in response to CpG-A and CpG-B

CpG-A forms aggregates that are retained in the early endosome and induce TLR9 activation there. This activation notably leads to the production of type-I-IFNs via IRF7. CpG-B in contrast, reaches late endosomes and triggers TLR9 there, leading to the production of DC maturing cytokines and chemokines. [3]

A variety of PS-ODNs have different effects on TLR9 activation. PS-CpG-ODNs are traditionally divided into two types: CpG-A and CpG-B. CpG-A-ODNs are hybrid ODNs, which are PD in the central portion containing the CpG-motif and PS at the 5'and 3' ends to stabilize this ODN. CpG-A has been reported to strongly activate IFN-α production in plasmacytoid dendritic cells (pDCs), but only weakly activates B cells. CpG-B, in contrast, poorly activates pDCs, but strongly stimulates B cells. This ODN entirely consists of PS linkages. [6] G-stretches in CpG-A are responsible for the formation of large aggregates that result in INF-production. [23] These aggregates retain in early endosomes and induce strong IRF-7 signaling. In contrast, CpG-B quickly reaches late endosomes where it induces

pDC maturation. (Fig 2.) [24, 25] In 2003 CpG-C was developed, which is a PS-ODN consisting of features of both CpG-B and CpG-A that is capable of strongly inducing pDCs as well as B cells, though lacking the aggregate formation-inducing G-stretches. [26] Finally, even antagonistic effects have been reported for some non-CpG PS-ODNs. [27, 28] While all types of ODNs can bind to TLR9, only binding of "activating" ODNs induces conformational changes that allow dimerization and thus activation of TLR9. [29]

ROLE OF HMGB1

One accessory protein for TLR9 has been reported thus far. This protein, HMGB1, is a common nuclear protein with various functions in facilitating protein binding to DNA, remodeling chromatin, transcription regulation, and V(D)J-recombination. [30, 31] Immune cells secrete HMGB1 in response to activation by for example LPS or stimulatory cytokines such as IFNγ. [32] Also necrosis passively releases HMGB1 in the extracellular environment. [33] The effect of CpG-ODN on the secretion of HMGB1 is unclear. [34, 35]

The reported roles for HMGB1 are diverse and include both stimulatory and suppressive activities. Ivanov *et al.* showed acquired HMGB1 from the environment binds to CpG-ODN in a CpG-motif- and sequence-specific fashion and enhances TLR9-dependent cytokine secretion. [35] HMGB1 was furthermore reported to bind to another receptor, RAGE, in absence of CpG-ODN and even better when bound to CpG-A ODNs. When bound to CpG-B ODNs, the interaction of HMGB1 with RAGE was decreased. After HMGB1-CpG-A-mediated engagement of TLR9, RAGE was reported to interact with TLR9 itself. The involvement of HMGB1 and RAGE in TLR9-mediated IFN-α production was further supported using DNA-immune complexes of Systemic Lupus Erythromatosus patients. [36] The interaction of HMGB1, RAGE, and TLR9 could act as a signaling complex that possibly mediates the observed HMGB1-mediated recruitment of TLR9 from the ER to endolysosomes. [35] In contrast, Popovic et al. reported that HMGB1-CpG-A, B, and C complexes inhibit DC maturation and IFN-α production. [37]

SIGNAL TRANSDUCTION

Roughly two signaling pathways are known for TLRs: the MyD88-dependent pathway and the TRIF-dependent pathway. Thus far, only TLR 3 and TLR 4 have been proven to signal

via the TRIF-dependent pathway. TLR9 exclusively signals via the MyD88-dependent pathway and induces a Th1-type of immune response.

The cytosolic part of TLR9 contains its signaling domain. This domain is a conserved signaling module called Toll/Interleukin-1 Receptor (TIR) domain. TLR-specific adaptor molecules such as TRIF and MyD88 also possess TIR domains through which they homotypically interact with the TLR via TIR-TIR interactions. These interactions have been proven essential for TLR-mediated signaling.

Each of the TLRs that have thus far been co-crystallized with their respective ligands showed dimer formation within the crystal. (Fig 3.) Dimerization of TLRs upon ligand binding is essential for TLR signaling. TLR dimerization can be both homo- and heterodimerization, which further expands the potential variety of molecular patterns that can be recognized by TLRs. Conformational changes that occur upon ligand binding likely affect the cytosolic domain such that the recruitment of signaling proteins such as MyD88 is facilitated. Also, since the active form of the signaling protein MyD88 is a homodimer, ligand-induced dimerization may aid in positioning the TIR domains of the TLR such that two MyD88 molecules are brought within the proper range and orientation of each other to facilitate the generation of an MyD88 dimer.

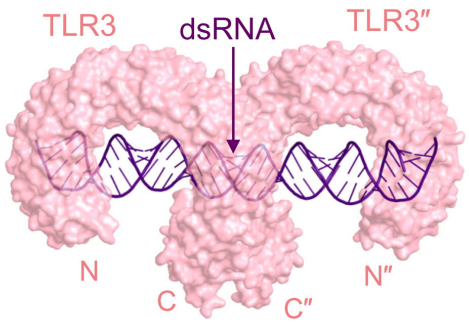

Figure 3: TLR3 ectodomain dimer

A spacefill structure based on crystallography data of the TLR3 ectodomain dimer in complex wit hits ligand dsRNA. The structure of TLR9 is likely similar to this structure. Dimerization of TLR monomers is required to make the TLR capable of signalling. [2]

Aside of the TIR domain, MyD88 also possesses a death-domain, which is a homotypic interaction motif that serves as a scaffold to recruit IL-1 receptor-associated kinases (IRAKs). IRAK-4 was shown to be essential for TLR9-dependent activation of IFN production. [38] This kinase activates IRAK-1 which in its turn activates TRAF6, another important protein in TLR signaling. TRAF6 is a key regulator in the activation of MAP kinase pathways and the release of IKKβ. MAP kinases such as JNK and p38 are important for the

activation of the transcription factor AP-1. IKKβ is released through phosphorylation of NEMO by TAK1, and in its turn phosphorylates IκB. Ubiquitinylation of this protein upon phosphorylation releases NF-κB from IκB by proteasomal degradation and allows NF-κB to be translocated into the nucleus and act as a transcription factor. IKKα is released along with IKKβ and activates an alternative NF-κB pathway by phosphorylating p100RelB. P100RelB is next processed into p52RelB and translocated to the nucleus where it acts as a transcription factor for chemokines in particular.

AP-1 and NFκB are both transcription factors for pro-inflammatory cytokines that are activated by TLRs in general. Since gene-expression is the result of a cooperation between all the transcription factors that are expressed in the cell, further fine tuning of the effects of TLR activation differs between cell types. However, also the TLRs introduce an extra fine-tuning step which is TLR-dependent. This is covered for by the interferon regulatory factors (IRFs). TLR9 has been reported to activate IRF-7 and IRF-1. IRAK-4-dependent TLR9-mediated IFN induction was reported as redundant for most viruses, [38] which emphasizes the importance of IRFs in TLR9-triggered type-I-IFN production since IRF activation is independent of IRAKs. The choice of signaling pathway used depends on the type of endosomal compartment from which is signaled. Early endosomes mostly produce IRF-induction, while late endosomes primarily lead to MAPK induction. [24, 25]

ROLE OF TLR9 IN IMMUNE REGULATION: PLASMACYTOID DCS TAKE CENTER STAGE

Activation of immune cells through TLR9 is important for defense against bacteria, DNA viruses and for autoimmune diseases. [3] Key players in TLR9-mediated immune responses are pDCs and B cells. pDCs are very potent producers of type-I-IFNs in response to viral infection. Interestingly, they selectively express the nucleic acid sensing TLRs 7 and 9, but not other TLRs. [39] pDCs that lack the expression of MyD88 or TLR9 lose the ability to produce IFN-α in response to stimulation with CpG-ODN. [40]

The roles of pDC type-I-IFN production were excellently reviewed by Gilliet *et al.* in 2008. [3] pDCs provide a link between the innate sensing of nucleic acids and the adaptive immune reaction. pDC-produced type-I-IFNs direct myeloid DCs (mDCs) to mature into antigen-presenting cells in response to HIV, and to stimulate antiviral T-cell responses to

herpes simplex virus. They increase the capability for cross presentation, boost CD8+ T cell expansion, and induce the differentiation of naïve T cells into Th1 cells. Finally, type-I-IFN secretion by pDCs activates NK cells and aids B cells to mature into plasma cells. [3] With such vast and potent effects of activation, negative regulation is important. To this purpose, pDCs encode several negatively regulating receptors. [3] Also, pDCs are reported capable of inducing the development of regulatory T cells (Treg) [41] and reverting anergic Treg cells back to active. [42] In contrast, the direct stimulation of Treg cells themselves via TLR9 seems to limit the conversion of Treg cells in the intestinal environment. [43]

In B cells, TLR9 stimulation has been reported to be important for isotype switching of antibody production [44] and providing co-stimulatory signals for the development of natural antibodies. [45]

TLR9 IN THE AUTOIMMUNE DISEASE SLE

Single nucleotide polymorphisms (SNPs) are gene variants with a frequency of 1% or higher within a population. Thus far, 20 SNPs have been identified for human TLR9, of which four are relatively common. [46] A small study reported the presence of a C on position 1237 to be correlative to asthma development in Europeans[46], but two other studies could not confirm a link of TLR9 with atopy. [47, 48] Interestingly, TLR9 polymorphisms have also been reported to play roles in the autoimmune diseases ulcerative colitis, [49] Crohn's disease, [50] and primary billiary cirrhosis. [51] Especially the link with the autoimmune disease SLE has received much scientific attention.

SYSTEMIC LUPUS ERYTHROMATOSUS

SLE is a systemic autoimmune reaction to self-derived nuclear antigens. These components are normally shielded from the immune system, but are released by necrosis. SLE patients respond with a type III hypersensitivity reaction, in which self-antibodies are raised to histone proteins, DNA, and other nuclear components. Binding of these antigens results in the formation of large antibody/antigen networks, called immune complexes. These get stuck in small vascular beds, most notably of the kidney, and induce a strong innate immune activation, resulting in severe damage of the surrounding tissue.

The role of TLR9 in Systemic Lupus Erythromatosus (SLE) is under heavy investigation. While de Jager *et al.* [52], Hur *et al.* [53], and Demirci *et al* [54]. proclaimed that common TLR9 polymorphisms do not affect the susceptibility to SLE, Tao *et al.* [55] reported that a G at position +1174, an intron of the TLR9 mRNA, predisposes for the development of SLE in a Japanese population. TLR9 expression was reported to be normal on pDCs, but up-regulated on peripheral B cells of SLE patients. [56] Also the numbers of B cells and peripheral blood mononuclear cells (PBMCs) that express TLR9 are increased in SLE. [57] In mice, induction of SLE can be done independently from TLR9. [58] Although these results suggest that TLR9 is not the cause of SLE, the protein is still involved in the disease.

pDCs were reported to be activated by DNA - antibody immune complexes of human SLE patients via TLR9 in combination with FcγRIIa. (Fig 5.)[59] In a mouse model for SLE, TLR9 responds to chromatin immune complexes using FcγRIII. [60] IFN-alpha serum levels are generally elevated in SLE and correlate to disease severity and activity. [61] Furthermore, IFN-alpha mRNA levels as well as TLR7 and TLR9 mRNA levels are elevated in PBMCs in correlative amounts. [62]

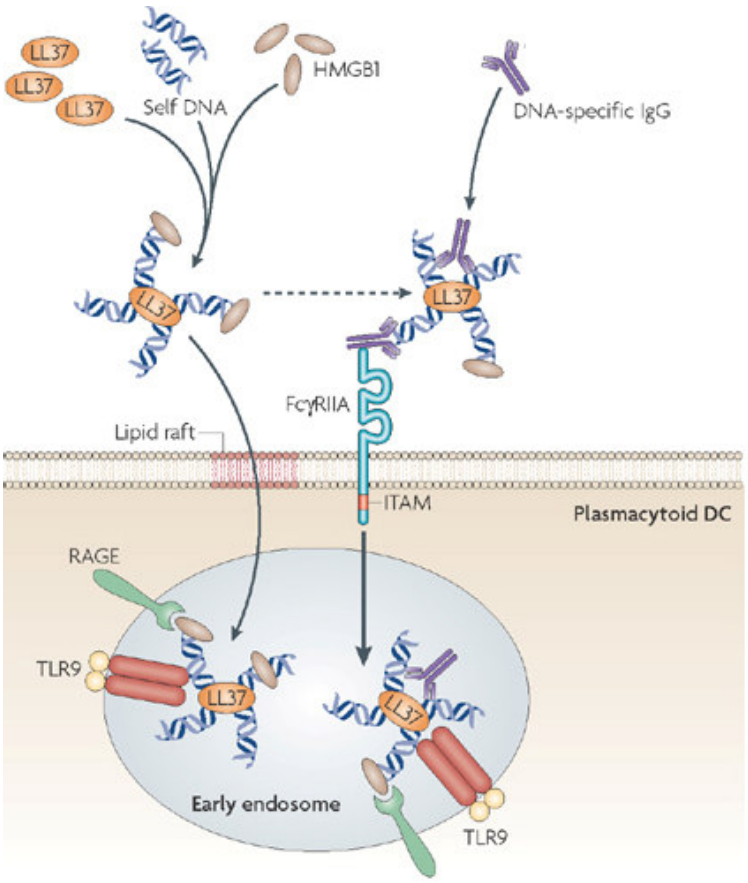

Figure 4: A model for TLR9 activation in SLE.

A novel model for TLR9 activation in SLE, based on recent data. LL37 is a defensin which has been reported to aggregate DNA. Production of this defensin and binding of HMGB1 from necrotic cells to optimizes DNA for recognition by TLR9. Engagement of RAGE along with TLR9 enhances the activation of TLR9. [3]

Several studies have suggested that TLR9 plays an important role in the production auto-antibodies. Ehlers *et al.* reported that TLR9 is important for the class switch of auto-antibodies to IgG2a/2b which they stated to be the pathogenic antibody class in SLE. [63] Similar data was reported by Christensen *et al*. [64] , Nakano *et al.* [65] and Thibault *et al.*[66] In contrast, Lartigue *et al.* found that TLR9 is essential for the development of anti-nucleosome antibodies in a mouse model, but does not affect anti-DNA antibodies. [67] Key in the development of auto-antibodies in SLE seems to be the synergistic engagement of DNA-containing antigens by the B cell receptor and TLR9. [68, 69] It was recently reported that the B cell receptor in complex with its DNA-containing antigen is internalized into

autophagosomes and recruits TLR9 to these vesicles, leading to hyperactivation of the MAP kinase pathway. [70]

Interestingly, mice lacking TLR7 have an improved SLE disease outcome, [64] while mice lacking TLR9 show an exacerbation of disease. [58, 64] This suggests that TLR9 has a dual role in SLE by acting both as an activator and as a suppressor of disease. That TLR7 expression in B cells of SLE patients has been reported to be dependent on TLR9 activation, [66] further adds to the theory of TLR9 as a suppressor of SLE.

DISCUSSION

Over the past half a decade, the knowledge of Toll-like receptor 9 has vastly expanded and evolved. Especially during the past two years a wealth of new data has been created.

Shockingly, it was shown that CpG-motifs were not responsible for TLR9 activation by DNA. Instead, it now seems that TLR9 has a broad range of specificity for DNA in general. This model is much simpler, and by that a lot more complicating for the host. After all, our own DNA can also be recognized by TLR9 and thus lead to nasty autoimmune diseases.

In attempt to prevent our bodies from being in an autoimmune state, TLR9 was evolutionally forced to internal vesicles to operate from there. A separate transport mechanism was developed for TLR 3, 7, and 9 which further limits the accessibility of these receptors to their ligands. HMGB1 and RAGE are possibly required to activate the transport of TLR9 to endolysosomes. Proteolytic cleavage is furthermore required in those compartments to activate the signaling capabilities of TLR9. The current data suggests that upon activation of the cell, TLR9 gets transported from the ER to endolysosomal compartments in an UNC93B-dependent fashion. From there, the processed TLR9 travels to the Golgi-complex. UNC93B is unlikely to play a role in the latter step. The importance of TLR9 trafficking to the Golgi is yet to be discovered.

To further prevent activation by self-DNA, our bodies express a lot of DNAse in the extracellular environment. That the strict handling of TLR9 is not fully capable of preventing autoimmune disease however is shown in SLE. By means of internalization of immune complexes of self-DNA containing antigens, self-DNA gains access to TLR9-containing endolysosomal compartments and can thus lead to auto-activation. In SLE, TLR9 activation is important in the development of pathogenic auto-antibodies to DNA-containing antigens by B cells. Also it seems to play a role in pDC-mediated type-I-IFN production, although data from knockout mice suggests that the role of TLR9 is more to control the activation rather than boost it.

The recently acquired knowledge that CpG-motifs are not required for activation of TLR9 is a revolution in understanding immunological processes involving TLR9 activation, such as

SLE. The abundance of information that was acquired using PS-ODNs however has not lost its value. PS-CpG-ODNs will still be important tools in TLR9 research because of their stability and ability to robustly and specifically activate this receptor. Furthermore, the generation of PS-ODNs with activating and suppressive effect as well as different activation patterns has provided a variety of tools for therapeutic and vaccine purposes .

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