Toll-like receptors and immune regulation: their direct and indirect modulation on regulatory CD4$^+$ CD25$^+$ T cells

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Summary

Regulatory CD4$^+$ CD25$^+$ T (Treg) cells with the ability to suppress host immune responses against self- or non-self antigens play important roles in the processes of autoimmunity, transplant rejection, infectious diseases and cancers. The proper regulation of CD4$^+$ CD25$^+$ Treg cells is thus critical for optimal immune responses. Toll-like receptor (TLR)-mediated recognition of specific structures of invading pathogens initiates innate as well as adaptive immune responses via antigen-presenting cells (APCs). Interestingly, new evidence suggests that TLR signalling may directly or indirectly regulate the immunosuppressive function of CD4$^+$ CD25$^+$ Treg cells in immune responses. TLR signalling may shift the balance between CD4$^+$ T-helper cells and Treg cells, and subsequently influence the outcome of the immune response. This immunomodulation pathway may therefore have potential applications in the treatment of graft rejection, autoimmune diseases, infection diseases and cancers.

Keywords: Toll-like receptors; regulatory CD4$^+$ CD25$^+$ T cells; immune response; immune tolerance; autoimmune disease

Introduction

The family of Toll-like receptors (TLRs) is a major class of receptors that recognize molecular patterns associated with pathogens including bacteria, viruses, fungi and protozoa. It was commonly accepted that TLR-mediated recognition of specific structures of invading pathogens initiates innate as well as adaptive immune responses via dendritic cells (DCs) or other antigen-presenting cells (APCs).[1–3] However, there is emerging evidence that TLR signalling participates in inflammation and immune responses that are driven by self-, allo- or xeno-antigens.[4–7] TLR signalling has been demonstrated to be involved in the immune recognition of allo- or xenografts and the occurrence of autoimmunity both in experimental and in clinical studies.[5,8–10] This observation was strongly supported by the expression of TLRs on almost all immune cells and the endogenous expression of their ligands on mammalian cells.

However, there has recently been an explosion of renewed interest in regulatory T (Treg) cells, especially on three postulated CD4$^+$ Treg cell populations: the naturally occurring and inducing CD4$^+$ CD25$^+$ Treg cells, and two inducible populations, type 1 regulatory T (Tr1) cells and type 3 regulatory T (Th3) cells.[11–13] It is clear now that the CD4$^+$ CD25$^+$ Treg cell population not only critically contributes to the maintenance of self-tolerance but also has the potential to prevent the immune rejection of allografts.[14,15] Recent studies have shown that TLR signalling may directly or indirectly regulate the immunosuppressive function of CD4$^+$ CD25$^+$ Treg cells in graft rejection, autoimmune diseases, infectious diseases and cancers,[16–19] which is the focus of the present review.

Abbreviations: APC, antigen-presenting cell; DC, dendritic cell; DD, death domain; Foxp3, Forkhead box protein 3; IFN, interferon; IRAK, IL-1R-associated kinase; IRF-3, interferon regulatory factor 3; IL, interleukin; LPS, lipopolysaccharide; LTA, lipoteichoic acid; MyD88, myeloid differentiation primary response protein 88; Mal, MyD88-adaptor-like; NF-κB, nuclear factor κB; PAMP, pathogen-associated molecular pattern; TGF, transforming growth factor; TNF, tumour necrosis factor; Treg cell, regulatory T cell; Tr1 cell, type 1 regulatory T cell; TRAF6, tumour necrosis factor receptor-associated factor 6; TRAM, TRIF-related adaptor molecule; TIRAP, TIR-associated protein; TRIF, TIR domain containing adaptor inducing IFN-β; Th3 cell, type 3 regulatory T cell; TLR, Toll-like receptor.

TLRs and their ligands

TLRs have recently emerged as a critical component of the innate immune system for detecting microbial infections, APC activation and the induction of adaptive immune responses.[20,21] At least 13 TLRs have been identified in humans and mice to date.[20,22–24] The initial role of TLRs in innate immunity against micro-organisms has been studied extensively.[25–31] They recognize limited but highly conserved molecular structures, so-called pathogen-associated molecular patterns (PAMPs) (Table 1).[22,32–34] Thus, TLRs recognize conserved molecular features of bacteria, fungi and viruses.

Interestingly and importantly, some endogenous ligands of TLRs were identified recently. Heat shock proteins (hsps) such as hsp60, hsp70, hsp90 and glycoprotein 96 (gp96; the endoplasmic reticulum form of hsp90) of bacterial and mammalian origins have been shown to induce the inflammatory response.[35] Using macrophages from C3H/HeJ mice with a *TLR4* gene point mutation, researchers demonstrated that the effects of recombinant human hsp60 (rhhsp60) were dependent on TLR4, suggesting that hsp60 might be an endogenous TLR4 ligand.[22,30,36] Since then, using *TLR4* mutant mice, *TLR2* knockout mice, and/or fibroblasts transfected with *TLR2*, *TLR3* or *TLR4* cDNA, researchers have found that fibrinogen, surfactant protein A, fibronectin extra domain A, heparan sulphate, soluble hyaluronan and murine β-defensin 2 are endogenous ligands for TLR4; hsp60, hsp70, gp96 and high mobility group 1 (HMGB1) protein are endogenous ligands for both TLR2 and TLR4;[32,34,35,37] and mRNA is an endogenous ligand for TLR3[38] (Table 1).

The identification of endogenous ligands for TLRs raised the possibility that TLRs may be involved in self-tolerance and surveillance, which are critical for the occurrence of autoimmune diseases and cancers.[3,39] TLRs recognize not only the endogenous and exogenous molecules but also the degradation products of endogenous macromolecules such as heparan sulphate and polysaccharide fragments of hyaluronan, which indicate tissue injury, infection and/or tissue remodelling.[22,32–34,36,37] However, fibrinogen is normally present in the circulation at high concentrations.[35,37] Thus, monocytes, DCs and sinusoidal macrophages such as hepatic Kupffer cells and splenic macrophages are constantly exposed to fibrinogen. However, surfactant protein A is normally present in lung alveoli where alveolar macrophages reside.[39,40] Life-long continuous exposure of immune cells to these TLR ligands may be potentially deleterious to the host. However, the question of why the recognition of endogenous molecules, such as fibrinogen and surfactant protein A, by TLR4 does not mediate the activation of APCs in a physical situation needs to be addressed.

Table 1. Toll-like receptors (TLRs) and their exogenous and endogenous ligands

TLR	Exogenous ligands	Endogenous ligands	References
TLR1	Bacterial triacyl lipopeptides and proteins in parasites	Unknown	22
TLR2	Bacterial diacyl lipopeptides, lipoteichoic acid from Gram-positive bacteria, and zymosan from the cell wall of yeast	hsp70, gp96, necrotic cells and HMGB1	22,29,30,32,34
TLR3	Double-stranded RNA from viruses	mRNA	20
TLR4	Endotoxin (LPS) from Gram- negative bacteria	hsp60, hsp70, hyaluronan, lung surfactant protein A, fibronectin, fibrinogen, heparan, HMGB1 and β-defensin 2	22,30,31,34
TLR5	Flagellin from mobile bacteria	Unknown	41
TLR6	Partnered with TLR2	Unknown	20
TLR7	Single-stranded RNA from viruses	Unknown	25
TLR8	Same as TLR7	Unknown	52
TLR9	CpG DNA from bacteria or viruses	Chromatin–IgG complexes	26
TLR10	Unknown	Unknown	17
TLR11	Profilin, a protein from the protozoan pathogen *Toxoplasmosis gondii* that can cause miscarriage; may also respond to components of bacteria that cause bladder and kidney infections	Unknown	22,27
TLR12	Unknown	Unknown	22,27
TLR13	Unknown	Unknown	20

gp96, glycoprotein 96 (the endoplamic reticulum form of hsp90); HMGB1, high- mobility group box 1; hsp, heat shock protein; IgG, immunoglobulin G; LPS, lipopolysaccharide.

TLR expression on T cells

TLR expression has been detected in many types of immune cells, including different subsets of DCs, T cells, neutrophils, eosinophils, mast cells, macrophages, monocytes and epithelial cells (Table 2).[22,41,42] Importantly, the expression of TLRs is related to the functional states of different subtypes of T cells. Studies have shown that naïve $CD4^+$ T cells do not express significant levels of TLR2 and TLR4 mRNA and intracellular proteins. Only a few $CD3^+$ T cells express TLR1, TLR2 or TLR4 on the cell surface when they have not been activated.[43] However, activated/memory T cells express appreciable levels of cell surface TLR2 and TLR4.[5,21,44] T-cell receptor (TCR) stimulation by cross-linked anti-CD3 monoclonal antibody (mAb) induces cell surface expression of TLR2 and TLR4 on naïve human and murine $CD4^+$ T cells.[5,45] By contrast, TCR stimulation significantly down-modulates surface TLR5 expression on human $CD4^+$ T cells.[41] Moreover, TLR3, TLR6, TLR7 and TLR9 are also expressed on $CD4^+$ T cells.[46] Thus, some TLRs may function as costimulator receptors for antigen-specific T-cell development and immune responses, and participate in the maintenance of T-cell memory.[47,48] These data indicate that pathogens, via their PAMPs, may contribute directly to the perpetuation and activation of memory T cells.

Some TLRs are expressed on $CD4^+$ $CD25^+$ Treg cells (see Table 4 below). It has been reported that $CD4^+$ $CD25^+$ Treg cells in naïve mice selectively express TLR4, TLR5, TLR7 and TLR8, whereas TLR1, TLR2, TLR3 and TLR6 appear to be more widely expressed on $CD4^+$ T cells, and not confined to $CD4^+$ $CD25^+$ Treg cells.[49,50] The distinct expression patterns of TLRs on $CD4^+$ $CD25^+$ Treg cells support the potential involvement of these TLRs in the regulation of $CD4^+$ $CD25^+$ Treg cells.

Table 2. The expression pattern of Toll-like receptors (TLRs) on different immune cells

TLR	Expression on immune cells	References
TLR1	Most cell types including DCs and B cells	22
TLR2	PMLs, DCs, monocytes and T cells	21,29,30
TLR3	DCs, NK cells and T cells	20,38
TLR4	Macrophages, DCs and T cells	20,30,31
TLR5	Monocytes, DCs, NK cells and T cells	20,41
TLR6	High expression in B cells and DCs; low in monocytes and NK cells	20,63
TLR7	B cells, DCs, monocytes and T cells	25,38
TLR8	Monocytes, DCs; low in NK and T cells	26,52
TLR9	DCs, B cells, PMLs, macrophages, NK cells and microglial cells	22,26
TLR10	B cells; low in DCs	17,38
TLR11	Unknown	22,27
TLR12	Unknown	22,27
TLR13	Unknown	20

DC, dendritic cell; NK, natural killer; PML, peripheral mononuclear leucocyte.

TLRs, $CD4^+$ $CD25^+$ Treg cells and the immune response

Naturally occurring and antigen-induced $CD4^+$ $CD25^+$ Treg cells have been extensively studied in mice and humans. Depletion of the naturally occurring subset of $CD4^+$ $CD25^+$ Treg cells results in various types of autoimmune disease.[11,12,27,41] $CD4^+$ $CD25^+$ Treg cells inhibit a wide range of autoimmune and inflammatory manifestations such asgastritis, oophoritis, orchitis, thyroiditis, inflammatory bowel disease and spontaneous autoimmune diabetes.[11,12,27,41,51] Although $CD4^+$ $CD25^+$ Treg cells play a critical role in the regulation of immunity, we know little about the regulation of $CD4^+$ $CD25^+$ Treg cells. Interestingly, new evidence suggests that TLR signalling may directly or indirectly regulate the immunosuppressive function of $CD4^+$ $CD25^+$ Treg cells in immune responses.[16,17]

To date, differential TLR expression on $CD4^+$ T cells and $CD4^+$ $CD25^+$ Treg cells has been found, suggesting that TLRs may be directly involved in adaptive immune responses, but how TLRs directly regulate adaptive immune responses is still not fully understood.[52,53] Recently, a close relationship between TLRs and autoimmune diseases has been reported in mouse models. In a mouse model of systemic lupus erythematosus (SLE), when TLR9 was absent, SLE was exacerbated with increased activation of lymphocytes and plasmacytoid DCs as well as enhanced levels of immunoglobulin G (IgG) and interferon-α (IFN-α) in sera. In contrast, TLR7-deficient mice had ameliorated SLE with decreased lymphocyte activation and serum IgG levels.[54] These data reveal opposing inflammatory and regulatory roles for TLR7 and TLR9 in SLE. Importantly, it is interesting that, in *in vitro* $CD4^+$ $CD25^+$ Treg cell suppression assays, engagement of TLR4 or TLR9 on freshly isolated mouse splenic DCs could significantly abrogate the immunosuppressive function of $CD4^+$ $CD25^+$ Treg cells, by rendering effector T cells resistant to $CD4^+$ $CD25^+$ Treg cell-mediated suppression through DC expression of interleukin-6 (IL-6), but not the effects of costimulation.[39] Thus, the regulation of TLRs on $CD4^+$ $CD25^+$ Treg cells may directly or indirectly play a role in the occurrence of autoimmune diseases.

There is accumulating evidence of significant alterations in TLR expression on T cells in patients who have infectious diseases, although it is unclear whether altered TLR expression on T cells contributes directly to pathophysiology or immune defence in these patients.[55,56] Furthermore, cross-talk between TLRs and $CD4^+$ $CD25^+$ Treg

Table 3. Potential roles of Toll-like receptors (TLRs) as therapeutic targets in inflammatory diseases, cancers and autoimmune diseases

TLR	Related diseases	References
TLR1	Bacterial/fungal diseases, Gram-positive sepsis	20,22
TLR2	Bacterial/fungal diseases, Gram-positive sepsis	21,29,30
TLR3	Viral diseases	10,20
TLR4	Bacterial diseases, Gram-negative sepsis, chronic inflammation, autoimmune diseases, cancers, atherosclerosis	20,30
TLR5	Bacterial diseases	41
TLR6	Mycobacterial diseases	3,20
TLR7	Viral diseases	20,38
TLR8	Viral diseases	20,52
TLR9	Bacterial and viral diseases, autoimmune diseases, cancers	26
TLR10	Unknown	27
TLR11	Unknown	22,27
TLR12	Unknown	22,27
TLR13	Unknown	20,52

cells in tumours has also been noted.[5,21] Elucidation of TLR signalling in adaptive immune responses in terms of both direct and indirect regulation of the immunosuppressive function of $CD4^+$ $CD25^+$ Treg cells may therefore have therapeutic benefits in inflammation, cancers and autoimmune diseases (Table 3).

The direct regulatory effects of TLRs on $CD4^+$ $CD25^+$ Treg cells

$TLR2^{-/-}$ mice, unlike $TLR4^{-/-}$ mice, contain significantly fewer $CD4^+$ $CD25^+$ Treg cells than control mice.[21] Administration of TLR2 ligands to wild-type mice results in significantly increased $CD4^+$ $CD25^+$ Treg cell numbers.[5,21] In the presence of a TLR2 agonist, such as the synthetic bacterial lipoprotein Pam3Cys-SK4, $CD4^+$ $CD25^+$ Treg cells expand markedly but their immunosuppressive function is temporarily abrogated.[5] However, studies have shown that hsp60 could act as a costimulator of $CD4^+$ $CD25^+$ Treg cells.[21,46,57] Treatment of $CD4^+$ $CD25^+$ Treg cells with hsp60 or its peptide p277 before anti-CD3 mAb-induced activation significantly enhanced the ability of the $CD4^+$ $CD25^+$ Treg cells to down-regulate the function of $CD4^+CD25^-$ or $CD8^+$ target T cells, detected through the inhibition of target T-cell proliferation and IFN-γ and tumour necrosis factor (TNF)-α secretion.[58] The enhanced costimulatory effects of hsp60 on $CD4^+$ $CD25^+$ Treg cells involved innate signalling via TLR2, which led to activation of protein kinase C, phosphatidyl-inositol-3-kinase, and p38.[58] hsp60-treated $CD4^+$ $CD25^+$ Treg cells suppressed target T cells both by cell contact mechanisms and by secretion of cytokine transforming growth factor (TGF)-β and IL-10.[58] Thus, hsp60, a self-molecule, can down-regulate adaptive immune responses by up-regulating $CD4^+$ $CD25^+$ Treg cells innately through TLR2 signalling and not through APCs. Thus, the effect of TLR2 on $CD4^+$ $CD25^+$ Treg cells is somewhat controversial and further investigation is required.

Researchers observed that exposure of $CD4^+$ $CD25^+$ Treg cells to the TLR4 ligand lipopolysaccharide (LPS) induced up-regulation of several activation markers and enhanced their survival or proliferation.[49] The proliferative response does not require APCs and is augmented by TCR triggering and IL-2 stimulation.[49] Most importantly, LPS treatment increases the immunosuppressive ability of $CD4^+$ $CD25^+$ Treg cells by 10-fold.[49] Moreover, LPS-activated $CD4^+$ $CD25^+$ Treg cells can efficiently control the occurrence of naïve $CD4^+$ effector T cell-mediated diseases.[49] These findings provide the first evidence that

Table 4. Toll-like receptor (TLR) expression and function on $CD4^+$ $CD25^+$ Treg cells

			Effects on $CD4^+$ $CD25^+$ Treg cells		
TLR	Effector T cells	$CD4^+CD25^+$ Treg cells	Cell proliferation	Suppression	References
TLR1	+	+			49
TLR2	+	+	?	Up-regulation/ down-regulation (?)	21,58
TLR4	+	++	Yes	Up-regulation	49
TLR5	+	++	No	Up-regulation	41,60
TLR6	+	+			49
TLR7	+	++			49
TLR8	+	++		Down-regulation	48
TLR9	+	+	Yes	Blocking (partially via effector T cells)	65

+, normal expression; ++ higher expression compared with effector T cells.

CD4+ CD25+ Treg cells respond directly to pro-inflammatory bacterial products, a mechanism that is likely to contribute to the control of inflammatory responses. However, others failed to observe effects of LPS on CD4+ CD25+ Treg cells.[41,45,59] Thus, LPS-induced signalling on CD4+ CD25+ Treg cells is still controversial.

TLR5 ligand flagellin has important effects in regulating mucosal immune responses.[41,60,61] Both human CD4+ CD25+ Treg cells and CD4+CD25− T cells express TLR5 at levels comparable to those on monocytes and DCs. Costimulation of CD4+ effector T cells with anti-CD3 mAb and flagellin resulted in enhanced proliferation and production of IL-2 at levels equivalent to those achieved by costimulation with CD28. In contrast, co-stimulation with flagellin did not break the hyporesponsiveness of CD4+ CD25+ Treg cells, but rather potently increased their immunosuppressive capacity and enhanced expression of Forkhead box protein 3 (Foxp3).[41,60] However, in a tumour mouse model, administration of flagellin, which is specifically recognized by TLR5, at 8–10 days after tumour implantation produced significant inhibition of the growth of the antigenic tumour, which was associated with an increased IFN-γ:IL-4 ratio and a decreased frequency of CD4+ CD25+ Treg cells.[62] In contrast, flagellin administered at the time of tumour implantation led to accelerated tumour growth, which was associated with a decreased IFN-γ:IL-4 ratio and an increased CD4+ CD25+ Treg cell frequency.[62] Whether the contrasting effects of activation of TLR5 by flagellin on tumour growth are attributable to the different impacts on effector T cells and CD4+ CD25+ Treg cells and/or the different effects on naïve and activated/memory CD4+ CD25+ Treg cells has not yet been determined.

In addition, TLR8 could directly reverse the immunosuppressive function of CD4+ CD25+ Treg cells.[21,52] It has been reported that CpG-A and poly (G10) oligonucleotides could directly reverse the immunosuppressive function of CD4+ CD25+ Treg cells in the absence of DCs, but the exact functional ingredients were not identified in that study.[49] Further experiments indicated that short poly (G) oligonucleotides [poly (G2), poly (G3) and poly (G4) with phosphorothioate linkages] had a more potent ability to reverse CD4+ CD25+ Treg cell function than longer oligonucleotides [poly (G5), poly (G7) and poly (G10)].[52] Interestingly, when TLR8 and myeloid differentiation primary response protein 88 (MyD88) were knocked down using an RNA interference method, the response ability of CD4+ CD25+ Treg cells to poly (G) oligonucleotides was abolished. Consistent with these results, TLR8 was consistently expressed by naturally occurring as well as inducing CD4+ CD25+ Treg cells.[52,63,64] A recent study more clearly demonstrated that the immunosuppression of CD4+ CD25+ Treg cells is abrogated by TLR8 triggering directly on the CD4+ CD25+ Treg cells, not on the CD4+ effector T cells.[52] These results consistently support the hypothesis that the TLR8–MyD88 signalling pathway directly controls the immunosuppressive function of CD4+ CD25+ Treg cells without the involvement of APCs.

The TLR9 ligand CpG oligodeoxynucleotide synergizes with anti-CD3 mAb to induce proliferation of both rat CD4+ CD25− and CD4+ CD25+ Treg cells.[65] Surprisingly, TLR9 ligand partially abrogates the suppressive activity mediated by CD4+ CD25+ Treg cells, which is partially attributable to the direct effect of TLR9 ligand on effector T cells which are rendered more resistant to the regulation exerted by CD4+ CD25+ Treg cells.[65] Thus, TLR9 ligand may rapidly increase the host's adaptive immunity by expanding effector T cells and also by attenuating the suppressive activity mediated by CD4+ CD25+ Treg cells.

Taking these results together, it is obvious that CD4+ CD25+ Treg cells express certain TLRs. In general, TLR2(?), TLR8 or TLR9 ligation abrogates or reverses the immunosuppressive function of CD4+ CD25+ Treg cells, whereas TLR2(?), TLR4 or TLR5 ligation enhances CD4+ CD25+ Treg cell suppressive capacity (Table 4).

The mechanisms by which TLRs modulate the immunosuppressive ability of CD4+ CD25+ Treg cells are not clear. One explanation is that the up-regulation or down-regulation of *Foxp3* expression following stimulation by different TLRs may be related to the functional alteration of CD4+ CD25+ Treg cells. However, how TLR signalling affects *Foxp3* expression needs to be addressed. Another possibility to explain the abrogated immunosuppressive function but enhanced proliferative capacity of CD4+ CD25+ Treg cells after TLR stimulation is suggested by reports indicating that CD4+ CD25+ Treg cells rapidly lose their ability to inhibit proliferation after receiving strong activation signals. Experimental data support the idea that TLR2 on murine CD4+ CD25+ Treg cells might function as a strong costimulatory trigger.[5,37] We may speculate that some TLR-mediated signals force CD4+ CD25+ Treg cells into the proliferative pathway, which might be paralleled by the reversal of their immunosuppressive capabilities. However, TLR4 may induce CD4+ CD25+ Treg cell proliferation while enhancing the immunosuppressive ability of CD4+ CD25+ Treg cells, which does not support this speculation.

The indirect regulatory effects of TLRs on CD4+ CD25+ Treg cells

A central function of TLRs is to directly activate acute antimicrobial defence systems. TLRs are known to induce the production of antimicrobial proteins and peptides by various cell types, including cells of myeloid origin in the gut epithelium.[4,41,66] Activation of resident macrophages through TLRs also leads to production of various cytokines (IL-1, IL-6, TNF-α, etc.) and chemokines (monocyte chemotactic protein 1, etc.), which collectively orchestrate the acute inflammatory response to infections.[53,67]

DCs are pivotally positioned at the interface of innate and adaptive immunity. Pathogen recognition is mediated by TLRs which are expressed at high levels on the surface of DCs. Analysis of MyD88-deficient mice demonstrated the critical role of TLRs in DC maturation and induction of adaptive immune responses.[3,68,69] MyD88-deficient mouse DCs could not prime antigen-specific naïve T cells *in vitro*.[70] While antigen mixed with complete Freund's adjuvant (CFA), which contains several ligands for TLRs, leads to a robust immune response in wild-type mice, it fails to trigger T-cell proliferation and IFN-γ production in MyD88-deficient mice.[71,72] These data suggest that engagement of TLRs on DCs *in vivo* is required for DC maturation, secretion of cytokines and chemokines and antigen-presenting ability, which subsequently control the choice of the effector T cells of adaptive immune responses. TLR signalling in macrophages and DCs leads to secretion of IL-12 which skews the resultant $CD4^+$ effector T-cell response towards the T helper type 1 (Th1) phenotype.[23] Moreover, TLR triggering in APCs contributes to the adaptive immune response by indirectly controlling the immunosuppression of $CD4^+$ $CD25^+$ Treg cells.[53]

Some researchers found that the immunosuppressive function of $CD4^+$ $CD25^+$ Treg cells is critically dependent on immature DCs and is readily reversed by TLR-induced activation of DCs.[59] The potential responsiveness of $CD4^+$ $CD25^+$ Treg cells to IL-2 was increased by the co-operative effects of IL-6 and IL-1, both of which are produced by TLR-activated mature DCs.[5,58,73] The pro-inflammatory cytokines produced by TLR-activated mature DCs are required for reversal of $CD4^+$ $CD25^+$ Treg cell anergy, but whether they are required to overcome the immunosuppression of $CD4^+$ $CD25^+$ Treg cells is a matter of controversy.[39,59] However, cytokines secreted by APCs in response to TLR ligands are also important for $CD4^+$ effector T cells to overcome the immunosuppressive effects of $CD4^+$ $CD25^+$ Treg cells.[18,23,74]

Some studies showed that decreased immunosuppression of $CD4^+$ $CD25^+$ Treg cells on effector T cells was mediated by soluble factors produced by DCs in response to the TLR4 ligand LPS or the TLR9 ligand CpG oligonucleotide.[5,19,23] The activity of LPS-treated DC supernatants was specifically abrogated by neutralizing anti-IL-6 mAbs, thus indicating the significance of IL-6 in the control of $CD4^+$ $CD25^+$ Treg cells on $CD4^+$ $CD25^-$ effector T cells.[19] These conclusions were further supported by the observation that the supernatant of LPS-stimulated DCs from IL-$6^{-/-}$ knock-out mice was unable to reverse suppression of $CD4^+$ $CD25^+$ Treg cells.[75] However, recombinant IL-6 did not substitute for DC supernatant. This suggests that, in addition to IL-6, some factors, as yet undefined, are essential in this process. However, preincubation of $CD4^+$ $CD25^+$ Treg cells with the supernatant of LPS-activated DCs did not block the immunosuppressive capacity of $CD4^+$ $CD25^+$ Treg cells,[76] suggesting that the supernatant of LPS-activated DCs may render $CD4^+$ $CD25^-$ effector T cells refractory to suppression rather than blocking the immunosuppressive capacity of $CD4^+$ $CD25^+$ Treg cells.[23,59,76,77] This regulating network may allow $CD4^+$ $CD25^+$ Treg cells to maintain their function and prevent self-reactive T-cell activation during an ongoing immune response to infections and other damage stimuli.

Although some TLR signalling on DCs by CpG or LPS rendered $CD4^+$ effector T cells refractory to $CD4^+$ $CD25^+$ Treg cell-mediated suppression, TLR-induced mature DCs are also capable of stimulating the proliferation of $CD4^+$ $CD25^+$ Treg cells,[78,79] which may be beneficial for the efficient down-regulation of the immune response as soon as the pathogens have been cleared.

Perspectives and closing remarks

Recent studies have demonstrated that the immunosuppressive function of $CD4^+$ $CD25^+$ Treg cells can be regulated through TLR signalling. Various pathways, including the indirect route via APCs and their cytokine products as well as the direct effects of TLRs on $CD4^+$ $CD25^+$ Treg cells, may collectively contribute to the generation, expansion and function of $CD4^+$ $CD25^+$ Treg cells. Further studies are needed to clarify the molecular mechanisms for the regulation of $CD4^+$ $CD25^+$ Treg cells via TLRs.

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