Oncogene (2008) 27, 181–189

www.nature.com/onc

REVIEW

Toll-like receptors and immune regulation: implications for cancer therapy

R-F Wang, Y Miyahara and HY Wang

The Center for Cell and Gene Therapy and Department of Pathology and Department of Immunology, Baylor College of Medicine, Houston, TX, USA

Toll-like receptors (TLRs) function as pathogen pattern recognition molecules that sensor and initiate innate and adaptive immune responses against microbes and cancer cells. Recognition of pathogen-derived ligands by TLRs expressed on many types of cells, including dendritic cells and T cells, triggers the nuclear factor (NF)-κB and type-1 interferon pathways, leading to the production of proinflammatory cytokines that are essential in stimulating $CD4^+$ T cells to differentiate to T helper (Th) 1, Th2 Th17 and regulatory T (Treg) cells. Recent studies indicate that Treg cells play a critical role in suppressing immune responses and inducing immune tolerance to cancer and infectious diseases. Of particular interest, the human TLR8 signaling pathway is essential for reversing the suppressive function of Treg cells. Thus, TLRs regulate cancer immunity and tolerance through innate immune responses mediated by Treg, dendritic and other immune cells. In this review, we focus on the current understanding of TLRs and Treg cells with emphasis on their roles in cancer immunity. Related information on non-TLR immune receptors will be briefly discussed.
Oncogene (2008) **27,** 181–189; doi:10.1038/sj.onc.1210906

Keywords: Toll-like receptors; tumor immunology; cancer immunotherapy; immune tolerance; innate immunity; regulatory T cells

Introduction

Toll-like receptors (TLRs) have emerged as sensors that can detect a variety of invading pathogens and malignant cells, thus serving as a first line of defense against infectious diseases and cancer. Ligand recognition by TLRs triggers dendritic cells (DCs) and other antigen-presenting cells to secrete proinflammatory cytokines and to promote DC maturation programs for the induction of adaptive immune responses (Iwasaki and Medzhitov, 2004; Takeda and Akira, 2005). At least 12 TLRs have been identified in humans and mice. These type-1 integral membrane glycoproteins are characterized by extracellular domains containing variable numbers of leucine-rich repeat (LRR) motifs and a cytoplasmic Toll/interleukin (IL)-1R homology domain. They recognize a limited but highly conserved set of molecular structures, so-called pathogen-associated molecular patterns. In addition to extracellular pattern recognition receptors (that is, TLRs), several intracellular pattern recognition receptors have been found to be responsible for the recognition of invading viruses in the cytoplasm. Retinoic acid-inducible protein 1 (RIG-1) is an interferon (IFN)-inducible protein-containing caspase-activating and recruitment domains (CARDs) and an RNA helicase domain that functions as a cytoplasmic receptor for dsRNA (Yoneyama *et al*., 2004; Kato *et al*., 2005). Melanoma differentiation-associated gene 5 (*MDA5*), a homologue of *RIG-1*, has also been suggested as a cytoplasmic receptor for dsRNA (Akira *et al*., 2006).

Nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs, also called caterpillar) represent a large family of receptor proteins harboring an LRR domain for ligand sensing, an NOD and an initiating signal domain, such as CARD, PYRIN or baculovirus inhibitor of apoptosis repeat domains. These NLR proteins have been implicated in the recognition of bacterial components (Inohara *et al*., 2005; Martinon and Tschopp, 2005; Ting and Davis, 2005). Activation of such cytoplasmic receptors by invading pathogens including bacteria and viruses leads to the production of proinflammatory cytokines such as IL-1β. Thus, TLRs, NLRs and RIG-1-like receptors (RLRs) are critical in bridging innate and adaptive immune responses. Although the roles of NLRs and RLRs in tumor immunity remain unclear, TLRs play a critical role in stimulating DC maturation, antigen uptake and presentation, and the differentiation of $CD4^+$ T cells (T helper (Th)1, Th2 and Th17) and controlling the suppressive function of regulatory T (Treg) cells. Thus, they represent a potent means of modulating immune responses to cancer vaccines, a novel strategy now being evaluated in clinical trials.

Ligand recognition and expression patterns

Since the discovery of TLRs, both natural and synthetic ligands for these receptors have been identified. TLR2 recognizes peptidoglycan found in gram-positive bacteria;

Correspondence: Dr R-F Wang, The Center for Cell and Gene Therapy and Department of Pathology and Department of Immunology, Baylor College of Medicine, ALKEK Building, N1120, Houston, TX 77030, USA.
E-mail: Rongfuw@bcm.tmc.edu

TLR4 recognizes lipopolysaccharide, which is unique to gram-negative bacteria; while TLR3, TLR7, TLR8, TLR9, RIG-1 and MDA-5 recognize nucleic acids. TLR3 recognizes double-stranded RNA produced during viral infection, and TLR9 recognizes an unmethylated cytosine-phosphate-guanine (CpG) DNA motif of prokaryotic genomes and DNA viruses (Takeda and Akira, 2005). TLR7 and 8 recognize single-stranded viral RNAs or guanosine-related analogs (loxoribine and imidazoquinoline) (Diebold *et al*., 2004; Heil *et al*., 2004; Lund *et al*., 2004). Human TLR8 recognizes Poly-G3 oligonucleotides (Peng *et al*., 2005), while murine TLR8 is not functional (Jurk *et al*., 2002). Unlike TLR2, 4, 5 and 6, which are expressed on the cell surface, TLR3, 7, 8 and 9 reside in endosomal compartments (Hemmi *et al*., 2002; Jurk *et al*., 2002; Akira and Takeda, 2004). In particular, TLR7, 8 and 9 form an evolutionary cluster (Crozat and Beutler, 2004; Takeda and Akira, 2005). Their endosomal localization is thought to be crucial for providing self versus non-self discrimination. For RNA sensing in the cytoplasm, RIG-I was recently shown to recognize the 5′ end of certain viral RNA genomes, specifically, to uncapped 5′-triphosphate RNA (Hornung *et al*., 2006; Pichlmair *et al*., 2006). Such 5′-triphosphates are generally removed or modified during post-transcriptional RNA processing of host RNA species, thereby remaining invisible or silent to innate immunity and providing a structural basis for the distinction of viral RNA from abundant self RNA in the cytoplasm of virally infected cells. TLR11 has been found to be inactive in humans, but is functional in mice and recognizes profilin-like molecule from the protozoan parasite *Toxoplasma gondii* (Zhang *et al*., 2004; Yarovinsky *et al*., 2005).

Among the large number of NLR family members, NOD1 and NOD2 recognize bacteria-derived γ-D-glutamyl-meso-diaminopimelic acid and muramyl dipeptide (MDP), respectively. Ligand binding to NOD1 and NOD2 induces their oligomerization and recruits RIP2/RICK to the complex, leading to activation of the nuclear factor (NF)-κB pathway and the production of inflammatory cytokines (Inohara *et al*., 2005; Martinon and Tschopp, 2005; Ting and Davis, 2005; Akira *et al*., 2006). Recently, several groups demonstrated that NALP3 recognizes bacterial RNA, ATP and uric-acid crystals (Kanneganti *et al*., 2006; Mariathasan *et al*., 2006; Martinon *et al*., 2006; Sutterwala *et al*., 2006). Specific ligands for other NOD-LRR family members remain to be identified.

Toll-like receptor expression has been detected on many types of cells, including different subsets of DCs, macrophages, T cells, neutrophils, eosinophils, mast cells, monocytes and epithelial cells (Iwasaki and Medzhitov, 2004). Some TLRs have been reported to be expressed by T cells, such as Tregs (Peng *et al*., 2005, 2007; Sutmuller *et al*., 2006b).

Signaling pathways of TLRs and other innate immune receptors

The current model of TLR signaling pathways predicts that TLR1, 2, 5, 7, 8 and 9 use MyD88 as their sole receptor proximal adapter to transduce signals (Figure 1). Thus, MyD88 is essential for the signaling of most TLRs to MyD88-IRKA4 and other downstream molecules. After the MyD88-IRAK4-IRAK1 complex activates TNFR-associated factor 6 (TRAF6), a ubiquitination protein ligase (E3), together with a ubiquitination E2 enzyme complex consisting of UBC13 and UEV1A, the TRAF6 complex catalyses the formation of a K63-linked polyubiquitin chain on TRAF6 itself and on NEMO, a subunit of IκB kinase (IKK) complex. Meanwhile, the recruitment of transforming growth factor (TGF)-β-activated kinase 1 (TAK1) and its binding proteins (TAB1, TAB2 and TAB33) leads to the phosphorylation of IKK-β and mitogen-activated protein (MAP) kinase 6 and then the activation of NF-κB and MAP kinase pathways for the production of inflammatory cytokines. Recent studies also suggest that MyD88 may also interact with IRF5 and 7 for the induction of proinflammatory cytokines or type-I IFN (IFN-α and IFN-β) response (Honda *et al*., 2005a, b; Takaoka *et al*., 2005). TLR3 relies on a MyD88-independent, but TIR domain-containing adaptor inducing IFN-beta (TRIF)-mediated pathway for the production of IFN-β in response to pathogen recognition, while TLR4 is linked to both MyD88- and TRIF-dependent pathways (Akira and Takeda, 2004; Takeda and Akira, 2005). Activation of TLR3 leads to recruitment of receptor-interacting protein 1, TRAF3 and TRAF6, which activates TRAF family member-associated NF-κB activator-binding kinase 1 (TBK1) and/or inducible IκB kinase (IKK-i), which directly phosphorylate IRF3 and IRF7 for the production of type-I IFN cytokines (Akira *et al*., 2006).

The adapter molecule IPS-1/MAVS/VISA/CARDIF of the RIG-1/MDA5 receptor for cytoplasmic dsRNA has been independently identified by several groups (Kawai *et al*., 2005; Meylan *et al*., 2005; Seth *et al*., 2005; Xu *et al*., 2005b). MAVS is present in the outer mitochondrial membrane, and the cleavage of this protein at the C terminus by NS3/4A protease of hepatitis C virus inactivates its ability to transduce signaling to TBK1 and IKK-i (Meylan *et al*., 2005; Seth *et al*., 2005; Lin *et al*., 2006).

Activation of NALP3 recruits apoptosis-associated speck-like protein containing a CARD (ASC) through a homotypic interaction between the PYRIN domains. ASC then recruits caspase 1 via its CARD, leading to the activation of caspase-1 (Martinon and Tschopp, 2004). Active caspase-1 then cleaves pro-IL-1β for release from cells. It appears that TLR signaling is required for the production of pro-IL-1β. Hence, signaling via both TLRs and NLRs pathways is needed to achieve the maximal production of IL-1β (Mariathasan and Monack, 2007).

Innate immunity links inflammation and cancer

Increasing evidence obtained with several different experimental approaches and tumor models supports the concept of cancer immunosurveillance (Dunn *et al*.,

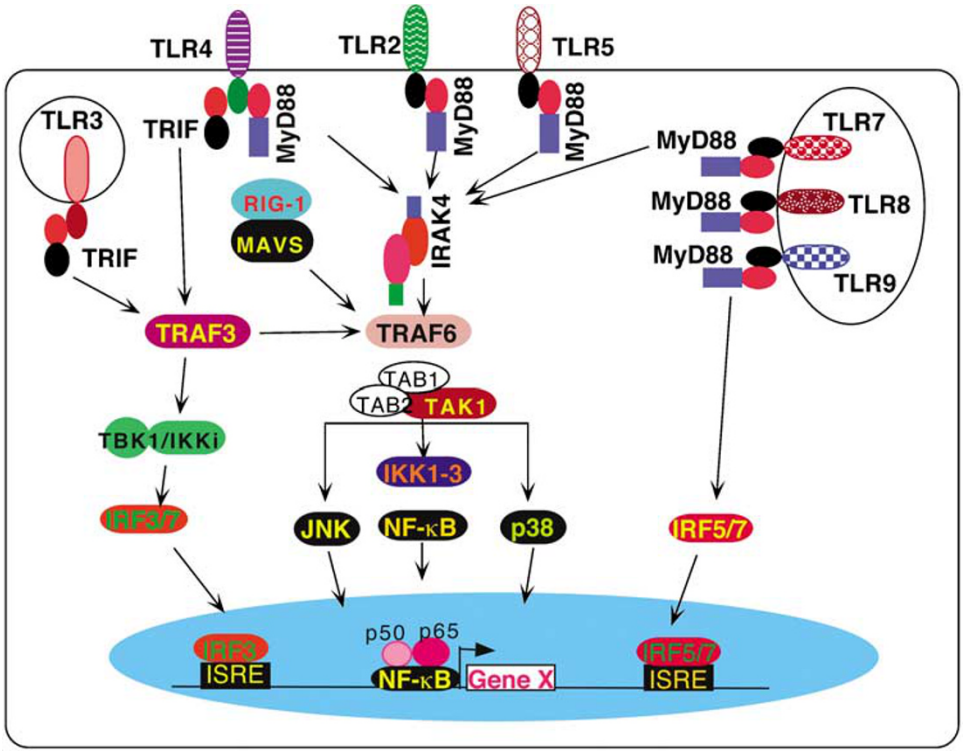

Figure 1 The signaling pathways of Toll-like receptors (TLRs) and RLRs. TLR2, TLR4 and TLR5 are expressed in the cell surface, while TLR3, TLR7, TLR8 and TLR9 are located in the endosomes. Retinoic acid-inducible protein 1 (RIG)-1 is a cytoplasmic receptor. TLR8 is functional in humans, but not functional in stimulating the nuclear factor (NF)-κB pathway in mice. The signaling pathways described are based on the data obtained from mice.

2004). Innate immune cells including natural killer (NK), natural killer T (NKT) and γδ T cells play a critical role in protecting the host against cancer (Dunn *et al*., 2004). Macrophages and DCs, in particular, function as major sensors of invading pathogens and transformed cells via a limited number of germ line-encoded pattern recognition receptors such as TLRs. Adaptive immunity is crucial to the elimination of pathogens and tumor cells in the late phase of host defense responses and generates more specific tumor immunity and immunological memory. Several recent studies demonstrated that the recruitment of tumor-infiltrating immune cells, in particular $CD8^+$ T cells, is important correlate of patient survival (Zhang *et al*., 2003; Sato *et al*., 2005; Galon *et al*., 2006), while the presence of other immune cells, such as Treg cells, is associated with a poor prognosis (Curiel *et al*., 2004). These studies clearly demonstrate a role for innate and adaptive immunity in cancer immunosurveillance.

Paradoxically, activation of the innate immune response (in particular the NF-κB pathway) through TLR-mediated recognition of invading pathogens can also promote tumor development depending upon the cytokine milieu and the presence or absence of other immune cells (Greten *et al*., 2004; Karin and Greten, 2005). For example, IL-1, IL-6, IL-8 and TGF-β released by immune cells and tumor cells promote angiogenesis, tumor growth and the differentiation of Th1, Th17 and Treg cells. Recent studies show that TLR and IL-1 receptor-associated kinases (IRAK) sequence polymorphism is an important risk factor for prostate cancer (Sun *et al*., 2005; Xu *et al*., 2005a). Chronic inflammation induced by *Helicobacter pylori* infection is the leading cause of stomach cancer, while inflammatory bowel diseases including ulcerative colitis and Crohn's disease are closely associated with colon cancer (Coussens and Werb, 2002; Karin *et al*., 2006). Similarly, hepatitis B and C viral infections are the leading factor contributing to liver cancer (Coussens and Werb, 2002; Karin *et al*., 2006). Recent studies clearly demonstrate that the MyD88-dependent signaling pathway has a critical role in the production of IL-6 and in both spontaneous and carcinogen-induced development of tumors (Naugler *et al*., 2007; Rakoff-Nahoum and Medzhitov, 2007). Taken together, these studies indicate that TLR-mediated inflammation from bacterial and viral infection can promote the development of cancers. Indeed, the blockade of signaling pathways required for inflammation or the induction of proinflammatory cytokines decreases the risk of tumorigenesis in model systems.

$CD4^+$ Th17 and Treg cells in cancer

Growing evidence suggests that $CD4^+$ Th cells play a central role in initiating and maintaining immune responses against cancer (Wang, 2001). Recent studies

further demonstrate that $CD4^+$ Th effector cells are critical for the subsequent expansion of memory $CD8^+$ T cells (Janssen *et al*., 2003; Shedlock and Shen, 2003; Sun and Bevan, 2003). In particular, $CD4^+$ Th cells are required during the maintenance phase of long-lived $CD8^+$ memory T cells (Sun *et al*., 2004). Regulation of tumor-necrosis factor-related apoptosis-inducing ligand expression in $CD8^+$ T cells accounts for the role of $CD4^+$ T cells in the generation and expansion of memory and memory-like $CD8^+$ T cells (Janssen *et al*., 2005; Hamilton *et al*., 2006). However, the presence of $CD4^+$ Treg cells and the newly discovered IL-17-producing T cells (Th17) may change our thinking about the role of $CD4^+$ T cells in cancer and many other diseases. Treg cells can significantly suppress immune responses, thus inducing immune tolerance at tumor sites, while Th17 cells have been linked to autoimmune diseases and cancer (Langowski *et al*., 2006; Weaver *et al*., 2006).

These $CD4^+$ Th cells comprise both Th1 and Th2 T-cell subsets based on their distinct cytokine secretion profiles. $CD4^+$ Th1 cells secrete cytokines such as IL-2 and IFN-γ. Specific TLR ligands expressed by bacteria and viruses may trigger DCs to secrete IL-12, which either stimulate NK cells to secrete IFN-γ or activate the Stat4 signaling pathway in naive T cells to secrete IFN-γ. IFN-γ produced by NK or naive T cells after IL-12 stimulates expression of Stat1 and subsequently T-bet, a master transcription factor in Th1 cells. $CD4^+$ Th1 cells provide cytokines for $CD8^+$ T cells and critical help through the activation of DCs, which are essential in the priming of $CD8^+$ T-cell responses. Many TLR ligands or agonists promote Th1 cell development, whereas some TLR ligands such as helminthes promote the differentiation of $CD4^+$ Th2 cells. IL-4 produced by basophils, esinophils and NKT cells initiates Stat6 signaling, leading to expression of GATA-3, a master transcription factor in Th2 cells (Weaver *et al*., 2006). Analysis of several hundreds of T-cell clones from tumor-infiltrating T cell lines established from melanoma patients revealed that over 95% of T cells are Th1 effectors, secreting IFN-γ and IL-2, but not IL-4 or IL-13 (R-F Wang, unpublished data).

Th17 cells

IL-17-producing T (Th17) cells are a distinct lineage within the general category of $CD4^+$ Th cells, secreting a unique set of cytokines IL-17 (Harrington *et al*., 2005; Park *et al*., 2005). TGF-β and IL-6 produced by tumor cells, Treg cells and APCs activate TGF-β and Stat3 signaling pathways, leading to expression of RORγt, a critical transcription factor for Th17 cells (Ivanov *et al*., 2006). Although Th17 cells are responsible for the pathogenesis of many autoimmune diseases, originally thought to be caused by self-reactive $CD4^+$ Th1 cells, their role in cancer is less clear. Nonetheless, both IL-17 and IL-23 have been identified in cancer tissues, suggesting that Th17 cells or proinflammatory cytokines may provide a tumor-promoting environment for cancer development or growth. We recently identified elevated percentages of Th17 cells in ovarian cancer tissues (R-F Wang, unpublished data).

Regulatory T cells

$CD4^+$ Treg cells have been identified as a small subset (5–6%) of the overall $CD4^+$ T-cell population. Besides naturally occurring $CD4^+$ $CD25^+$ Treg cells, other $CD4^+$ Treg cells include Tr-1 cells secreting IFN-γ and IL-10, and Th3 cells secreting high levels of TGF-β, IL-4 and IL-10 (Roncarolo and Levings, 2000; Weiner, 2001; Francois Bach, 2003). Foxp3 has proved to be a specific marker of $CD4^+$ Treg cells in both mice and humans (Fontenot *et al*., 2003; Hori *et al*., 2003; Khattri *et al*., 2003; Walker *et al*., 2003; Wang *et al*., 2004). Its expression is highly restricted to the subset of Treg cells and correlates with immune suppressor activity, irrespective of CD25 expression (Fontenot *et al*., 2005; Wan and Flavell, 2005).

$CD4^+$ Treg cells can profoundly suppress host immune responses and induce self-tolerance (Roncarolo and Levings, 2000; Sakaguchi *et al*., 2001; Shevach, 2002). Thus, despite their protective role in autoimmune diseases, these cells have inhibitory effects on cancer immunotherapy and infectious diseases (Shevach, 2000; Sakaguchi *et al*., 2001; Belkaid *et al*., 2002; Sakaguchi, 2004). Recent studies demonstrated an elevated proportion of $CD4^+$ $CD25^+$ Treg cells in the total $CD4^+$ T-cell populations in several different human cancers, including lung, breast and ovarian tumors (Woo *et al*., 2001; Liyanage *et al*., 2002; Curiel *et al*., 2004). In previous studies, we demonstrated the presence of antigen-specific $CD4^+$ Treg cells at tumor sites (Wang *et al*., 2004, 2005), and showed that Treg cells suppressed the proliferation of naive $CD4^+$ T cells and inhibited IL-2 secretion of $CD4^+$ effector cells upon activation by tumor-specific antigens (Wang and Wang, 2007). Finally, we recently identified $CD8^+$ Treg as well as γδ-TCR Treg cells in prostate and breast cancer (Kiniwa *et al*., 2007; Peng *et al*., 2007). The former cells expressed Foxp3 molecules, while the latter did not. Like $CD4^+$ Treg cells, both of these Treg cell subtypes mediate immune suppression and inhibit antitumor immunity.

Control of differentiation of Th17 and Treg cells by TLR-stimulated cytokines

To determine the factors with critical roles in driving Th17 cell differentiation, several groups demonstrated that TGF-β and IL-6, but not IL-23, are essential for Th17 cell commitment (Bettelli *et al*., 2006; Mangan *et al*., 2006; Veldhoen *et al*., 2006), although IL-23 may still be required for survival and maintenance of Th17 cells *in vivo*. Because TGF-β is an important factor in the development and conversion of Treg cells, it may also influence the fate of $CD4^+$ T cells to become Treg cells or Th17 cells. The transcriptional factor for Treg cell development, Foxp3, is upregulated in the presence of TGF-β, but is inhibited by IL-6 through still poorly defined mechanisms. Several groups recently reported

that IL-21 cooperates with TGF-β to promote Th17 cells and inhibit Treg cell differentiation (Korn *et al*., 2007; Nurieva *et al*., 2007; Zhou *et al*., 2007), while retinoic acid is capable of inhibiting the IL-6-driven induction of proinflammatory Th17 cells and promoting antiinflammatory Treg differentiation (Mucida *et al*., 2007).

Human Th17 cells were recently identified, but their regulation by cytokines differs from that in mice. For example, human Th17 cell differentiation requires IL-1β for differentiation, and IL-2, IL-6 and IL-23 for expansion of memory Th17 cells (Acosta-Rodriguez *et al*., 2007; Wilson *et al*., 2007). Importantly, TGF-β, an essential factor for murine Th17 cell differentiation, actually inhibits human Th17 cell differentiation (Acosta-Rodriguez *et al*., 2007). Despite the important role of Th17 in autoimmune diseases, its role in tumor development and progression is limited. One group recently reported that IL-23 promotes inflammatory responses and reduces $CD8^+$ T-cell responses (Langowski *et al*., 2006), while others claimed that DCs expressing IL-23 enhance antitumor immunity (Hu *et al*., 2006; Yuan *et al*., 2006). Hence, determining the subsets and prevalence of $CD4^+$ T cells in the tumor microenvironment could be critically important to improving therapeutic cancer vaccines. We recently demonstrated the presence of high percentages of human Th17 cells in ovarian cancer (R-F Wang, unpublished data). Moreover, ovarian tumor cells and tumor-associated fibroblasts secrete a large amount of IL-6, while tumor-associated APCs secrete large amounts of IL-1β and IL-6, both of which are critical for the differentiation and expansion of Th17 cells (R-F Wang, unpublished data). The cytokine milieu in the tumor microenvironment may be directly associated with TLR-mediated inflammation, thus determining whether $CD4^+$ T cells differentiate to Th1, Th2, Th17 and Treg cells. It appears that Th17 cell-mediated inflammation promotes tumor growth, while Treg cells inhibit antitumor immunity at tumor sites.

Functional control of Treg cell function by TLR signaling

To overcome immune suppression mediated by Treg cells, we screened cytokines and TLR ligands for their ability to reverse Treg-suppressive activity. We found that Poly-G10 oligonucleotides can directly reverse their suppressive function in the absence of DCs, depending upon a short stretch of guanosine oligonucleotides (2–10) (Figure 2). Using RNA interference technology, we showed that the TLR8-MyD88 signaling pathway is required for the reversal of Treg cell function by Poly-G oligonucleotides (Peng *et al*., 2005). Consistent with this result, we further showed that natural ligands for human TLR8, ssRNA40 and ssRNA33 derived from HIV viral sequences (Heil *et al*., 2004) completely reversed the suppressive function of Treg cells. These results suggest that binding and activation of TLR8 by either guanosine-containing DNA or RNA oligonucleotides is a key step in the reversal of Treg-suppressive function.

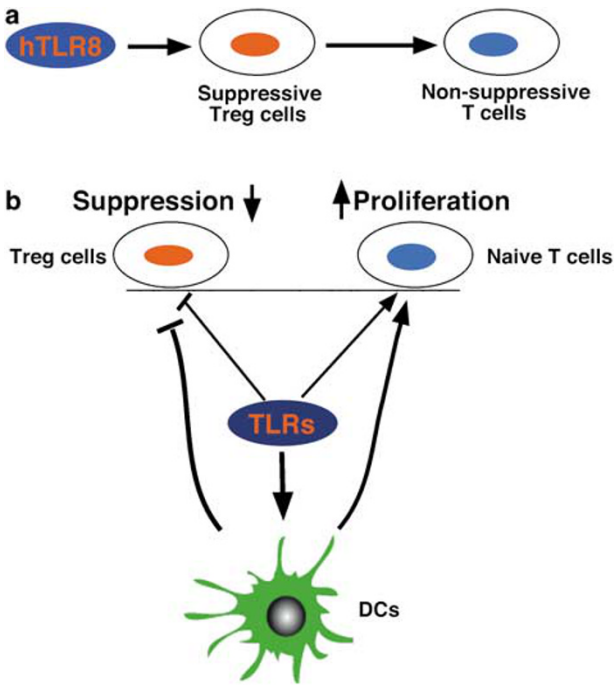

Figure 2 Control of regulatory T (Treg) cell-suppressive function by Toll-like receptors (TLRs) in humans and mice. (**a**) Human TLR8 directly controls the suppressive function of Treg cells. (**b**) TLRs control the balance between the suppressive function of murine Treg cells and naive T cell proliferation directly or indirectly.

Importantly, we found that ligands for other human TLRs could not reverse the suppressive function of Treg cells, suggesting that TLR8 activation is specifically linked to regulation of Treg cell function (Figure 2). This finding is consistent with the data of another group showing that falgellin-mediated activation of TLR5 on human DCs and effector T cells leads to enhanced proliferation and IL-2 secretion by these cells (Crellin *et al*., 2005). Stimulation of human Treg cells with flagellin increased rather than reversed their suppressive function (Crellin *et al*., 2005). Interestingly, Poly-G oligonucleotides could not reverse the suppressive activity of murine Treg cells (since TLR8 is not functional in mice; Jurk *et al*., 2002), although it is likely that other TLR ligands in mice affect Treg cell function and growth. Indeed, recent studies show that murine TLR2-deficient mice reduce the number of $CD4^+$ $CD25^+$ Treg cells (Netea *et al*., 2004). Activation of TLR2 with its ligand (Pam3Cys) directly increases the proliferation of murine Treg cells and transiently reverses their suppressive function (Sutmuller *et al*., 2006a). However, since several studies reported that functional regulation of murine Treg cells is mainly achieved by TLR via DCs (Pasare and Medzhitov, 2003; Kubo *et al*., 2004; Fehervari and Sakaguchi, 2004), further studies are needed to clarify this issue. Thus, the balance between murine Treg cell-suppressive function and naive/effector T-cell proliferation is controlled by TLRs through directly acting on Treg and effector T cells and indirectly acting on DCs (Figure 2).

TLR8-mediated reversal of Treg cell function can apply to antigen-specific as well as naturally occurring Treg cells. Beside different subsets of $CD4^+$ Treg cells, we recently identified $CD8^+$ Treg cells and γδ-TCR Treg cells in prostate and breast cancer (Kiniwa *et al*., 2007; Peng *et al*., 2007), and showed that they also express a low level of human TLR8 molecules. One of the important questions is whether TLR8 ligands including Poly-G3 and ssRNA40 can reverse the suppressive function of $CD8^+$ Treg cells and γδ-TCR Treg cells. Interestingly, we demonstrated that the suppressive function of $CD8^+$ Treg cells and γδ-TCR Treg cells can be reversed after Poly-G oligonucleotide treatment, suggesting that these cells share a common TLR8 signaling-mediated mechanism with previously characterized $CD4^+$ Treg cell subsets. Further dissection of the downstream pathways of TLR8-MyD88 in shRNA knockdown experiments revealed that TRAF6, p38, IKKα and IKKβ are required to enable Treg cells to respond to Poly-G3 treatment, while TAK1, JNK1 and extracellular signal-regulated kinase molecules are dispensable (Peng *et al*., 2007). These findings raise an intriguing possibility that manipulation of the TLR8 signaling pathway through its ligands or the use of drug inhibitors of key molecules in this pathway may allow one to block the suppressive function of different subsets of Treg cells, thus improving the efficacy of cancer vaccines.

Targeting TLRs for cancer therapy

Several TLR agonists have been developed as anticancer drugs. The TLR7 agonist imiquimod, for example, has been used to treat superficial basal cell carcinoma (Stockfleth *et al*., 2003), while theTLR9 agonist CpG-ODN B type is being evaluated in clinical trials in patients with melanoma and lymphoma (Speiser *et al*., 2005; Jahrsdorfer *et al*., 2005a). TLR4 agonists, including monophosphoryl lipid, have been used as adjuvant for vaccines against HBV and other pathogens (Baldridge *et al*., 2004). Several mechanisms have been proposed to explain the apparent adjuvant effects of TLR agonists on antitumor immunity. First, TLRs trigger the secretion of critical cytokines such as IL1, IL-6 and IL-12 by DCs, which are important for T-cell differentiation and the induction of potent adaptive immunity. Several groups have shown that conjugation of certain TLR ligands (that is, for TLR2, TLR4, TLR7 and TLR9) to peptides or proteins significantly enhances $CD4^+$ and $CD8^+$ T-cell responses compared with administration of TLR ligands or a peptide/protein mixture alone (Jackson *et al*., 2004; Wille-Reece *et al*., 2005; Blander and Medzhitov, 2006). Third, TLRs can directly stimulate the proliferation of $CD4^+$ and $CD8^+$ T cells as well as reverse the suppressive function of Treg cells (Crellin *et al*., 2005; Peng *et al*., 2005; Tabiasco *et al*., 2006). Finally, TLR9 and TLR3 agonists may also induce apoptosis of TLR-expressing tumor cells (Jahrsdorfer *et al*., 2005b; Salaun *et al*., 2006). However, as discussed above, optimal antitumor immunity requires robust enhancement of the effector T-cell response induced by tumor antigenic peptides and control or elimination of Treg cell-suppressive function. Thus, the combination of peptide-based vaccines with TLR agonists, in particular a TLR8 agonist, may greatly improve the therapeutic potential of cancer vaccines.

Conclusions and perspectives

Since their discovery a decade ago, TLRs have been shown to be critical for efficient innate and adaptive immunity and the framework of TLR-mediated signaling pathway has been elucidated. By contrast, the ligands recognized by NLRs and their signaling pathways remain to be determined. TLRs, NLRs and RLRs are clearly important in host response against infectious diseases and autoimmune diseases, the role of NLRs and RLRs in cancer immunosurveillance is much less certain. Increasing evidence indicates that immune suppression in the tumor microenvironment is a major obstacle to the development of effective therapeutic cancer vaccines. Tumor cells not only recruit Treg cells directly to tumor sites, but also help to convert naïve and/or effector T cells into Treg cells by providing antigenic stimulation and cytokines directly or indirectly through tumor-infiltrating innate immune cells. TLR- and NLR-dependent cytokine production by tumor cells, tumor-associated APCs and T cells is also critical in stimulating the differentiation of Th1, Th17 and Treg cells. The role of tumor-associated Th17 cells in cancer remains to be determined since inflammation has been linked to cancer. Recent studies from our own laboratory demonstrate that the suppressive function of different types of Treg cells can be reversed through the manipulation of TLR8 signaling; however, many questions remain to be addressed before the full clinical application of this observation is clear. For example, we are still uncertain as to why human TLR8 signaling can turn off the suppressive function of Treg cells, but other TLRs cannot, even though many of them use the same adapter molecules, such as MyD88 and IRAK4. Our most recent studies indicate that human TLR8 may use a unique signaling pathway to reverse Treg cell function. Thus, dissection of the TLR8-mediated signaling pathway and identification of the key molecules responsible for immune suppression are important challenges in the field. While many TLR agonists have been evaluated as anticancer drugs for cancer therapy, the therapeutic potential of reversing Treg cell function via TLR stimulation, either alone or in the combination with peptide-based vaccination, remains to be explored in the next few years.

Acknowledgements

This work is in part supported by grants from National Institutes of Health, American Cancer Society and Cancer Research Institute.

References

Acosta-Rodriguez EV, Napolitani G, Lanzavecchia A, Sallusto F. (2007). Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. *Nat Immunol* **8**: 942–949.

Akira S, Takeda K. (2004). Toll-like receptor signalling. *Nat Rev Immunol* **4**: 499–511.

Akira S, Uematsu S, Takeuchi O. (2006). Pathogen recognition and innate immunity. *Cell* **124**: 783–801.

Baldridge JR, McGowan P, Evans JT, Cluff C, Mossman S, Johnson D *et al*. (2004). Taking a Toll on human disease: Toll-like receptor 4 agonists as vaccine adjuvants and monotherapeutic agents. *Expert Opin Biol Ther* **4**: 1129–1138.

Belkaid Y, Piccirillo CA, Mendez S, Shevach EM, Sacks DL. (2002). CD4+CD25+ regulatory T cells control Leishmania major persistence and immunity. *Nature* **420**: 502–507.

Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M *et al*. (2006). Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* **441**: 235–238.

Blander JM, Medzhitov R. (2006). Toll-dependent selection of microbial antigens for presentation by dendritic cells. *Nature* **440**: 808–812.

Coussens LM, Werb Z. (2002). Inflammation and cancer. *Nature* **420**: 860–867.

Crellin NK, Garcia RV, Hadisfar O, Allan SE, Steiner TS, Levings MK. (2005). Human CD4+ T cells express TLR5 and its ligand flagellin enhances the suppressive capacity and expression of FOXP3 in CD4+CD25+ T regulatory cells. *J Immunol* **175**: 8051–8059.

Crozat K, Beutler B. (2004). TLR7: a new sensor of viral infection. *Proc Natl Acad Sci USA* **101**: 6835–6836.

Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P *et al*. (2004). Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med* **10**: 942–949.

Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C. (2004). Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science* **303**: 1529–1531.

Dunn GP, Old LJ, Schreiber RD. (2004). The immunobiology of cancer immunosurveillance and immunoediting. *Immunity* **21**: 137–148.

Fehervari Z, Sakaguchi S. (2004). Control of Foxp3+ CD25+CD4+ regulatory cell activation and function by dendritic cells. *Int Immunol* **16**: 1769–1780.

Fontenot JD, Gavin MA, Rudensky AY. (2003). Foxp3 programs the development and function of CD4(+)CD25(+) regulatory T cells. *Nat Immunol* **4**: 330–336.

Fontenot JD, Rasmussen JP, Williams LM, Dooley JL, Farr AG, Rudensky AY. (2005). Regulatory T cell lineage specification by the forkhead transcription factor foxp3. *Immunity* **22**: 329–341.

Francois Bach J. (2003). Regulatory T cells under scrutiny. *Nat Rev Immunol* **3**: 189–198.

Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C *et al*. (2006). Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* **313**: 1960–1964.

Greten FR, Eckmann L, Greten TF, Park JM, Li ZW, Egan LJ *et al*. (2004). IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell* **118**: 285–296.

Hamilton SE, Wolkers MC, Schoenberger SP, Jameson SC. (2006). The generation of protective memory-like CD8+ T cells during homeostatic proliferation requires CD4+ T cells. *Nat Immunol* **7**: 475–481.

Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM *et al*. (2005). Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* **6**: 1123–1132.

Heil F, Hemmi H, Hochrein H, Ampenberger F, Kirschning C, Akira S *et al*. (2004). Species-specific recognition of single-stranded RNA via Toll-like receptor 7 and 8. *Science* **303**: 1526–1529.

Hemmi H, Kaisho T, Takeuchi O, Sato S, Sanjo H, Hoshino K *et al*. (2002). Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. *Nat Immunol* **3**: 196–200.

Honda K, Ohba Y, Yanai H, Negishi H, Mizutani T, Takaoka A *et al*. (2005a). Spatiotemporal regulation of MyD88-IRF-7 signalling for robust type-I interferon induction. *Nature* **434**: 1035–1040.

Honda K, Yanai H, Negishi H, Asagiri M, Sato M, Mizutani T *et al*. (2005b). IRF-7 is the master regulator of type-I interferon-dependent immune responses. *Nature* **434**: 772–777.

Hori S, Nomura T, Sakaguchi S. (2003). Control of regulatory T cell development by the transcription factor foxp3. *Science* **299**: 1057–1061.

Hornung V, Ellegast J, Kim S, Brzozka K, Jung A, Kato H *et al*. (2006). 5'-Triphosphate RNA is the ligand for RIG-I. *Science* **314**: 994–997.

Hu J, Yuan X, Belladonna ML, Ong JM, Wachsmann-Hogiu S, Farkas DL *et al*. (2006). Induction of potent antitumor immunity by intratumoral injection of interleukin 23-transduced dendritic cells. *Cancer Res* **66**: 8887–8896.

Inohara N, Chamaillard M, McDonald C, Nunez G. (2005). NOD-LRR proteins: role in host–microbial interactions and inflammatory disease. *Annu Rev Biochem* **74**: 355–383.

Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ *et al*. (2006). The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17(+) T helper cells. *Cell* **126**: 1121–1133.

Iwasaki A, Medzhitov R. (2004). Toll-like receptor control of the adaptive immune responses. *Nat Immunol* **5**: 987–995.

Jackson DC, Lau YF, Le T, Suhrbier A, Deliyannis G, Cheers C *et al*. (2004). A totally synthetic vaccine of generic structure that targets Toll-like receptor 2 on dendritic cells and promotes antibody or cytotoxic T cell responses. *Proc Natl Acad Sci USA* **101**: 15440–15445.

Jahrsdorfer B, Muhlenhoff L, Blackwell SE, Wagner M, Poeck H, Hartmann E *et al*. (2005a). B-cell lymphomas differ in their responsiveness to CpG oligodeoxynucleotides. *Clin Cancer Res* **11**: 1490–1499.

Jahrsdorfer B, Wooldridge JE, Blackwell SE, Taylor CM, Griffith TS, Link BK *et al*. (2005b). Immunostimulatory oligodeoxynucleotides induce apoptosis of B cell chronic lymphocytic leukemia cells. *J Leukoc Biol* **77**: 378–387.

Janssen EM, Droin NM, Lemmens EE, Pinkoski MJ, Bensinger SJ, Ehst BD *et al*. (2005). CD4+ T-cell help controls CD8+ T-cell memory via TRAIL-mediated activation-induced cell death. *Nature* **434**: 88–93.

Janssen EM, Lemmens EE, Wolfe T, Christen U, Von Herrath MG, Schoenberger SP. (2003). CD4(+) T cells are required for secondary expansion and memory in CD8(+) T lymphocytes. *Nature* **421**: 852–856.

Jurk M, Heil F, Vollmer J, Schetter C, Krieg AM, Wagner H *et al*. (2002). Human TLR7 or TLR8 independently confer

responsiveness to the antiviral compound R-848. *Nat Immunol* **3**: 499.

Kanneganti TD, Ozoren N, Body-Malapel M, Amer A, Park JH, Franchi L *et al*. (2006). Bacterial RNA and small antiviral compounds activate caspase-1 through cryopyrin/Nalp3. *Nature* **440**: 233–236.

Karin M, Greten FR. (2005). NF-kappaB: linking inflammation and immunity to cancer development and progression. *Nat Rev Immunol* **5**: 749–759.

Karin M, Lawrence T, Nizet V. (2006). Innate immunity gone awry: linking microbial infections to chronic inflammation and cancer. *Cell* **124**: 823–835.

Kato H, Sato S, Yoneyama M, Yamamoto M, Uematsu S, Matsui K *et al*. (2005). Cell type-specific involvement of RIG-I in antiviral response. *Immunity* **23**: 19–28.

Kawai T, Takahashi K, Sato S, Coban C, Kumar H, Kato H *et al*. (2005). IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. *Nat Immunol* **6**: 981–988.

Khattri R, Cox T, Yasayko SA, Ramsdell F. (2003). An essential role for Scurfin in CD4(+)CD25(+) T regulatory cells. *Nat Immunol* **4**: 337–342.

Kiniwa Y, Miyahara Y, Wang HY, Peng W, Peng G, Wheeler TM *et al*. (2007). CD8+ Foxp3+ regulatory T cells mediate immune suppression in prostate cancer. *Clin Cancer Res* (in press).

Korn T, Bettelli E, Gao W, Awasthi A, Jager A, Strom TB *et al*. (2007). IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. *Nature* **448**: 484–487.

Kubo T, Hatton RD, Oliver J, Liu X, Elson CO, Weaver CT. (2004). Regulatory T cell suppression and anergy are differentially regulated by proinflammatory cytokines produced by TLR-activated dendritic cells. *J Immunol* **173**: 7249–7258.

Langowski JL, Zhang X, Wu L, Mattson JD, Chen T, Smith K *et al*. (2006). IL-23 promotes tumour incidence and growth. *Nature* **442**: 461–465.

Lin R, Lacoste J, Nakhaei P, Sun Q, Yang L, Paz S *et al*. (2006). Dissociation of a MAVS/IPS-1/VISA/Cardif-IK-Kepsilon molecular complex from the mitochondrial outer membrane by hepatitis C virus NS3-4A proteolytic cleavage. *J Virol* **80**: 6072–6083.

Liyanage UK, Moore TT, Joo HG, Tanaka Y, Herrmann V, Doherty G *et al*. (2002). Prevalence of regulatory T cells is increased in peripheral blood and tumor microenvironment of patients with pancreas or breast adenocarcinoma. *J Immunol* **169**: 2756–2761.

Lund JM, Alexopoulou L, Sato A, Karow M, Adams NC, Gale NW *et al*. (2004). Recognition of single-stranded RNA viruses by Toll-like receptor 7. *Proc Natl Acad Sci USA* **101**: 5598–5603.

Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO *et al*. (2006). Transforming growth factor-beta induces development of the T(H)17 lineage. *Nature* **441**: 231–234.

Mariathasan S, Monack DM. (2007). Inflammasome adaptors and sensors: intracellular regulators of infection and inflammation. *Nat Rev Immunol* **7**: 31–40.

Mariathasan S, Weiss DS, Newton K, McBride J, O'Rourke K, Roose-Girma M *et al*. (2006). Cryopyrin activates the inflammasome in response to toxins and ATP. *Nature* **440**: 228–232.

Martinon F, Petrilli V, Mayor A, Tardivel A, Tschopp J. (2006). Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature* **440**: 237–241.

Martinon F, Tschopp J. (2004). Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases. *Cell* **117**: 561–574.

Martinon F, Tschopp J. (2005). NLRs join TLRs as innate sensors of pathogens. *Trends Immunol* **26**: 447–454.

Meylan E, Curran J, Hofmann K, Moradpour D, Binder M, Bartenschlager R *et al*. (2005). Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. *Nature* **437**: 1167–1172.

Mucida D, Park Y, Kim G, Turovskaya O, Scott I, Kronenberg M *et al*. (2007). Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science* **317**: 256–260.

Naugler WE, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM *et al*. (2007). Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. *Science* **317**: 121–124.

Netea MG, Sutmuller R, Hermann C, Van der Graaf CA, Van der Meer JW, van Krieken JH *et al*. (2004). Toll-like receptor 2 suppresses immunity against *Candida albicans* through induction of IL-10 and regulatory T cells. *J Immunol* **172**: 3712–3718.

Nurieva R, Yang XO, Martinez G, Zhang Y, Panopoulos AD, Ma L *et al*. (2007). Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. *Nature* **448**: 480–483.

Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH *et al*. (2005). A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol* **6**: 1133–1141.

Pasare C, Medzhitov R. (2003). Toll pathway-dependent blockade of CD4+CD25+ T cell-mediated suppression by dendritic cells. *Science* **299**: 1033–1036.

Peng G, Guo Z, Kiniwa Y, Voo KS, Peng W, Fu T *et al*. (2005). Toll-like receptor 8 mediated-reversal of CD4+ regulatory T cell function. *Science* **309**: 1380–1384.

Peng G, Wang HY, Peng W, Kiniwa Y, Seo K, Wang R-F. (2007). Tumor-infiltrating gamma-delta T cells suppress T and dendritic cell function via mechanisms controlled by a unique Toll-like receptor signaling pathway. *Immunity* **27**: 334–348.

Pichlmair A, Schulz O, Tan CP, Naslund TI, Liljestrom P, Weber F *et al*. (2006). RIG-I-mediated antiviral responses to single-stranded RNA bearing 5′-phosphates. *Science* **314**: 997–1001.

Rakoff-Nahoum S, Medzhitov R. (2007). Regulation of spontaneous intestinal tumorigenesis through the adaptor protein MyD88. *Science* **317**: 124–127.

Roncarolo MG, Levings MK. (2000). The role of different subsets of T regulatory cells in controlling autoimmunity. *Curr Opin Immunol* **12**: 676–683.

Sakaguchi S. (2004). Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses. *Annu Rev Immunol* **22**: 531–562.

Sakaguchi S, Sakaguchi N, Shimizu J, Yamazaki S, Sakihama T, Itoh M *et al*. (2001). Immunologic tolerance maintained by CD25+ CD4+ regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. *Immunol Rev* **182**: 18–32.

Salaun B, Coste I, Rissoan MC, Lebecque SJ, Renno T. (2006). TLR3 can directly trigger apoptosis in human cancer cells. *J Immunol* **176**: 4894–4901.

Sato E, Olson SH, Ahn J, Bundy B, Nishikawa H, Qian F *et al*. (2005). Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. *Proc Natl Acad Sci USA* **102**: 18538–18543.

Seth RB, Sun L, Ea CK, Chen ZJ. (2005). Identification and characterization of MAVS, a mitochondrial antiviral

signaling protein that activates NF-kappaB and IRF3. *Cell* **122**: 669–682.
Shedlock DJ, Shen H. (2003). Requirement for CD4 T cell help in generating functional CD8 T cell memory. *Science* **300**: 337–339.
Shevach EM. (2000). Regulatory T cells in autoimmmunity. *Annu Rev Immunol* **18**: 423–449.
Shevach EM. (2002). CD4+ CD25+ suppressor T cells: more questions than answers. *Nat Rev Immunol* **2**: 389–400.
Speiser DE, Lienard D, Rufer N, Rubio-Godoy V, Rimoldi D, Lejeune F *et al*. (2005). Rapid and strong human CD8+ T cell responses to vaccination with peptide, IFA, and CpG oligodeoxynucleotide 7909. *J Clin Invest* **115**: 739–746.
Stockfleth E, Trefzer U, Garcia-Bartels C, Wegner T, Schmook T, Sterry W. (2003). The use of Toll-like receptor-7 agonist in the treatment of basal cell carcinoma: an overview. *Br J Dermatol* **149**(Suppl 66): 53–56.
Sun J, Wiklund F, Zheng SL, Chang B, Balter K, Li L *et al*. (2005). Sequence variants in Toll-like receptor gene cluster (TLR6-TLR1-TLR10) and prostate cancer risk. *J Natl Cancer Inst* **97**: 525–532.
Sun JC, Bevan MJ. (2003). Defective CD8 T cell memory following acute infection without CD4 T cell help. *Science* **300**: 339–342.
Sun JC, Williams MA, Bevan MJ. (2004). CD4+ T cells are required for the maintenance, not programming, of memory CD8+ T cells after acute infection. *Nat Immunol* **5**: 927–933.
Sutmuller RP, den Brok MH, Kramer M, Bennink EJ, Toonen LW, Kullberg BJ *et al*. (2006a). Toll-like receptor 2 controls expansion and function of regulatory T cells. *J Clin Invest* **116**: 485–494.
Sutmuller RP, Morgan ME, Netea MG, Grauer O, Adema GJ. (2006b). Toll-like receptors on regulatory T cells: expanding immune regulation. *Trends Immunol* **27**: 387–393.
Sutterwala FS, Ogura Y, Szczepanik M, Lara-Tejero M, Lichtenberger GS, Grant EP *et al*. (2006). Critical role for NALP3/CIAS1/cryopyrin in innate and adaptive immunity through its regulation of caspase-1. *Immunity* **24**: 317–327.
Tabiasco J, Devevre E, Rufer N, Salaun B, Cerottini JC, Speiser D *et al*. (2006). Human effector CD8+ T lymphocytes express TLR3 as a functional coreceptor. *J Immunol* **177**: 8708–8713.
Takaoka A, Yanai H, Kondo S, Duncan G, Negishi H, Mizutani T *et al*. (2005). Integral role of IRF-5 in the gene induction programme activated by Toll-like receptors. *Nature* **434**: 243–249.
Takeda K, Akira S. (2005). Toll-like receptors in innate immunity. *Int Immunol* **17**: 1–14.
Ting JP, Davis BK. (2005). CATERPILLER: a novel gene family important in immunity, cell death, and diseases. *Annu Rev Immunol* **23**: 387–414.
Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. (2006). TGFbeta in the context of an inflammatory cytokine milieu supports *de novo* differentiation of IL-17-producing T cells. *Immunity* **24**: 179–189.
Walker MR, Kasprowicz DJ, Gersuk VH, Benard A, Van Landeghen M, Buckner JH *et al*. (2003). Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4+CD25− T cells. *J Clin Invest* **112**: 1437–1443.
Wan YY, Flavell RA. (2005). Identifying Foxp3-expressing suppressor T cells with a bicistronic reporter. *Proc Natl Acad Sci USA* **102**: 5126–5131.
Wang HY, Deen AL, Guangyong P, Zhong G, Yanchun L, Yukiko K *et al*. (2004). Tumor-specific human CD4+ regulatory T cells and their ligands: implication for immunotherapy. *Immunity* **20**: 107–118.
Wang HY, Peng G, Guo Z, Shevach EM, Wang R-F. (2005). Recognition of a new ARTC1 peptide ligand uniquely expressed in tumor cells by antigen-specific CD4+ regulatory T cells. *J Immunol* **174**: 2661–2670.
Wang HY, Wang RF. (2007). Regulatory T cells and cancer. *Curr Opin Immunol* **19**: 217–223.
Wang R-F. (2001). The role of MHC class II-restricted tumor antigens and CD4+ T cells in antitumor immunity. *Trends Immunol* **22**: 269–276.
Weaver CT, Harrington LE, Mangan PR, Gavrieli M, Murphy KM. (2006). Th17: an effector CD4 T cell lineage with regulatory T cell ties. *Immunity* **24**: 677–688.
Weiner HL. (2001). Induction and mechanism of action of transforming growth factor-beta-secreting Th3 regulatory cells. *Immunol Rev* **182**: 207–214.
Wille-Reece U, Flynn BJ, Lore K, Koup RA, Kedl RM, Mattapallil JJ *et al*. (2005). HIV Gag protein conjugated to a Toll-like receptor 7/8 agonist improves the magnitude and quality of Th1 and CD8+ T cell responses in nonhuman primates. *Proc Natl Acad Sci USA* **102**: 15190–15194.
Wilson NJ, Boniface K, Chan JR, McKenzie BS, Blumenschein WM, Mattson JD *et al*. (2007). Development, cytokine profile and function of human interleukin 17-producing helper T cells. *Nat Immunol* **8**: 950–957.
Woo EY, Chu CS, Goletz TJ, Schlienger K, Yeh H, Coukos G *et al*. (2001). Regulatory CD4(+)CD25(+) T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer. *Cancer Res* **61**: 4766–4772.
Xu J, Lowey J, Wiklund F, Sun J, Lindmark F, Hsu FC *et al*. (2005a). The interaction of four genes in the inflammation pathway significantly predicts prostate cancer risk. *Cancer Epidemiol Biomarkers Prev* **14**: 2563–2568.
Xu LG, Wang YY, Han KJ, Li LY, Zhai Z, Shu HB. (2005b). VISA is an adapter protein required for virus-triggered IFN-beta signaling. *Mol Cell* **19**: 727–740.
Yarovinsky F, Zhang D, Andersen JF, Bannenberg GL, Serhan CN, Hayden MS *et al*. (2005). TLR11 activation of dendritic cells by a protozoan profilin-like protein. *Science* **308**: 1626–1629.
Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M *et al*. (2004). The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nat Immunol* **5**: 730–737.
Yuan X, Hu J, Belladonna ML, Black KL, Yu JS. (2006). Interleukin-23-expressing bone marrow-derived neural stem-like cells exhibit antitumor activity against intracranial glioma. *Cancer Res* **66**: 2630–2638.
Zhang D, Zhang G, Hayden MS, Greenblatt MB, Bussey C, Flavell RA *et al*. (2004). A Toll-like receptor that prevents infection by uropathogenic bacteria. *Science* **303**: 1522–1526.
Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G *et al*. (2003). Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *N Engl J Med* **348**: 203–213.
Zhou L, Ivanov II, Spolski R, Min R, Shenderov K, Egawa T *et al*. (2007). IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat Immunol* **8**: 967–974.