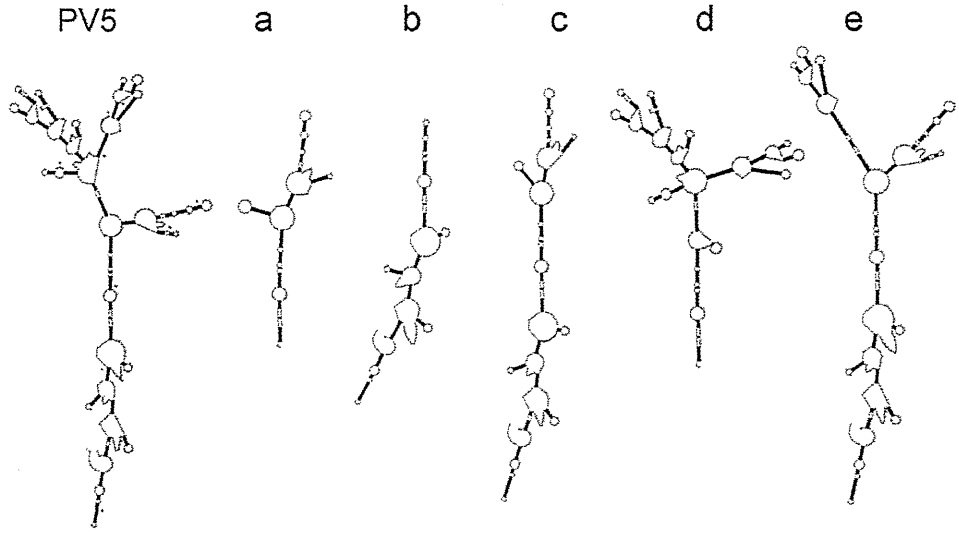

	PV5	a	b	c	d	e
length (nt)	630	191	250	367	380	469
stability: FBS- free medium	+	-	+	+	+	+
stability: FBS-containing medium	+	-	+	+	-	+
IFN-β promoter activity in HEK293 cells (FBS-free medium)	+	-	-	+	-	+
IFN-β production in mouse splenic DC (FBS-free medium)	++	-	+	++	+	++

Figure 1 RNA structures recognized by TLR3

PV5 and its derivatives (RNA a–e) were transcribed *in vitro* using PV cDNA as a template. Upper panel, secondary structure of PV5 and its derivatives (RNA a–e) predicted by the Mfold software. Thick lines indicate dsRNA regions (1–11 bp). RNAs were incubated in FBS-free or -containing medium at 37 °C for 30 min. The degradability of RNAs was assessed by electrophoresis on agarose gel. The TLR3-activating ability of RNAs was assessed by IFN-β promoter reporter assay with HEK-293 cells transiently expressing human TLR3 and IFN-β production from splenic DCs isolated from wild-type and TLR3-deficient mice in FBS-free medium [26]. All RNAs failed to induce IFN-β production in splenic DCs isolated from TLR3-deficient mice. IFN-β production in mouse splenic DCs. +, <150 pg/ml; + +, >150 pg/ml.

human epidermal keratinocytes via TLR3 [42]. UVB-damaged small nuclear RNAs, including U1 RNA (165 nt in length) were the determinants of TLR3 activation, but the precise mechanism underlying how UVB-damaged U1 RNA activates TLR3 is unknown.

The point of our recent study was that TLR3 recognizes incomplete stem structures formed in viral ssRNA and induces innate immune signalling [26]. Analyses with *in vitro* transcribed PV-derived ssRNAs and dsRNAs revealed that some PV ssRNAs activate TLR3 extracellularly, but do not activate RLRs, in human and mouse cells. Stability and length of RNA are crucial factors for TLR3 activation in that case. Functional PV RNA, 630 nt in length (PV5), bound to TLR3 ECD with high affinity, and both the N- and C-terminal dsRNA-binding sites of TLR3 ECD are required for PV5-induced IFN-β promoter activation in HEK (human embryonic kidney)-293 cells that transiently express human TLR3 (Figure 1). Furthermore, PV5 was internalized into cells via clathrin- and raftlin-mediated endocytosis and co-localized with endosomal TLR3, as observed previously with poly(I:C) uptake [43,44]. The secondary structure of PV5 predicted by Mfold software showed that PV5 possess double-strand regions (<11 bp in length) arranged in tandem, which are segmented with bulge or internal loops (Figure 1). The TLR3-activating ability of PV5 was abolished with RNaseIII treatment, indicating that the RNA duplex in PV5 is required for both the stability and functionality for the TLR3 activation. Analyses of PV5-derived RNAs partly having PV5 secondary structure (RNAs a–e in Figure 1) showed that longer stem structure with bulge and internal loops typically shown in RNA model c is the core RNA structure required for TLR3 activation in PV5 (Figure 1). Considering that dsRNA forms an A-type nucleotide duplex with 11 bp per turn [45], and that seven contiguous base pairs are needed for rapid duplex formation of DNA and RNA [46], incomplete stem structures containing contiguous base pairs may be required for stability that facilitates TLR3 binding. A fascinating model has been proposed for TLR3 dimer formation, in which shorter RNA duplexes (21–30 bp) can form less stable complexes with two TLR3 molecules [36]. Thus appropriate length or topology of multiple incomplete stems is required for TLR3 oligomerization, leading to the production of type I IFNs and pro-inflammatory

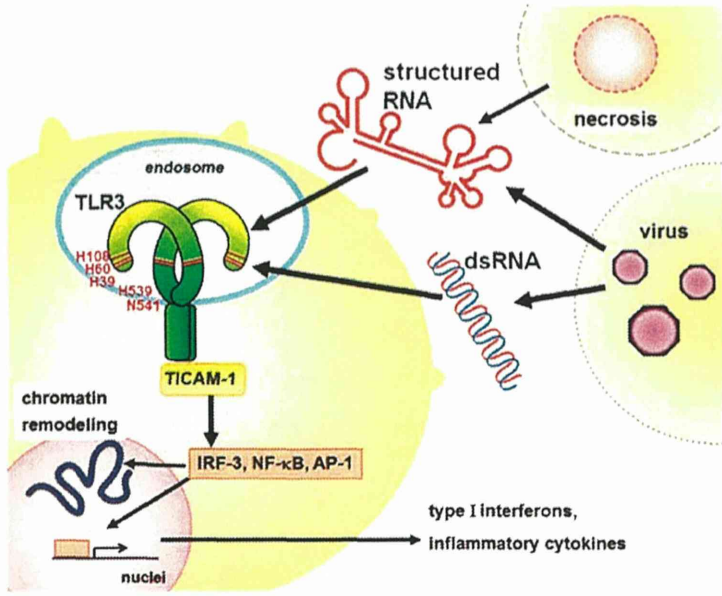

Figure 2 Model for dsRNA/structured RNA-induced TLR3-mediated immunity

Upon viral infection and sterile inflammation, virus- and host-derived RNAs are released from necrotic cells. In local environments, extracellular viral dsRNAs and virus/host-derived structured RNAs are rapidly taken up into cells via clathrin/raftlin-dependent endocytosis and delivered to endosomal TLR3. Once TLR3 is oligomerized by dsRNA/structured RNA, it recruits the adaptor protein TICAM-1 that activates the transcription factors, IRF-3, NF-κB and AP-1, leading to the production of type I IFNs and proinflammatory cytokines. The TLR3–TICAM-1 signal also induces chromatin modification in fibroblasts. In myeloid DCs, TLR3 activation triggers DC maturation capable of activating NK cells and CTLs. The key residues of TLR3, the N-terminal His^{39} in LRR-NT, His^{60} in LRR1, His^{108} in LRR3 and the C-terminal His^{539} and Asn^{541} in LRR20, which are involved in RNA binding are shown.

cytokines. RNA tertiary structure is also important for both the stability and activity of PV RNA.

Notably, mouse splenic DCs responded to shorter RNAs with mismatched duplexes that failed to activate human TLR3 expressed in epithelial cells, suggesting cell type- or species-specific RNA recognition by TLR3 (Figure 1). The precise mechanisms underlying this are currently unknown, but the high density of TLR3 expression and the potent phagocytic activity of mouse splenic DCs are advantageous for RNA-induced oligomerization of TLR3 and effective RNA uptake. In a study by Ewald et al. [47], mouse TLR3 was reported to undergo cathepsin-mediated proteolytic processing in the macrophage cell line RAW in a manner similar to that of mouse TLR9. Subsequent studies also demonstrated that human TLR3 ECD is cleaved at the loop exposed in LRR12 by cathepsins in a cell-type dependent manner [48,49], and the N- and C-terminal halves of human TLR3 remain associated after cleavage [49]. Requirement of proteolytic cleavage in TLR3 signalling appears to depend on cell type [49,50]. Potentially shorter structured RNAs may be recognized by protease-processed TLR3 in mouse DCs. Further studies are required to clarify the cell type- or species-dependent RNA recognition by TLR3.

UPTAKE OF exRNA

The ability of exRNAs to induce cellular responses primarily depends on the stability of these RNAs in the extracellular milieu and whether they are taken up into cells. dsRNA is resistant to degradation compared with ssRNA and, thus, viral dsRNA released from infected cells can be a potent activator of neighbouring virus-uninfected cells, leading to the induction of anti-viral states. Poly(I:C) is the most common dsRNA in both *in vitro* and *in vivo* studies to induce cellular responses, including type I IFN production and NK (natural killer) cell activation. Poly(I:C) is internalized into cells through clathrin-mediated endocytosis and delivered to endosomal TLR3 and to cytoplasmic MDA5 (melanoma differentiation-associated gene 5) [51]. Watanabe et al. [44] demonstrated that the cytoplasmic lipid raft protein raftlin is essential for poly(I:C) cellular uptake in human myeloid DCs and epithelial cells. In raftlin knockdown cells, surface-bound poly(I:C) neither enters the cells nor activates TLR3 and MDA5, indicating that cellular uptake is a pre-requisite for dsRNA-induced cellular responses. Upon poly(I:C) stimulation, raftlin translocates from the cytoplasm to the cell surface, where it associates with the clathrin–AP-2 (clathrin-associated adaptor protein-2) complex and induces cargo delivery. Interestingly, structured PV RNA is also internalized into cells via raftlin-mediated endocytosis and is delivered to endosomal TLR3 [26]. B- and C-type ODNs (oligodeoxynucleotides) that share the uptake receptor with poly(I:C) in humans inhibit cellular uptake of PV RNA [26,43,44,52]. Hence extracellular dsRNA/structured RNA and ODNs are recognized by a common uptake receptor and their internalization is regulated by raftlin. Mouse DCs express raftlin-2 in addition to raftlin, and raftlin knockdown does not affect poly(I:C) cellular uptake, suggesting that raftlin-2 functionally compensates for raftlin [44].

The uptake receptors for poly(I:C) have been identified by several groups. Lee et al. [53] reported that CD14 enhances poly(I:C)-induced TLR3 activation by mediating poly(I:C) uptake in mouse macrophages. Furthermore, the scavenger receptor class-A was identified as a cell surface receptor for dsRNA in human bronchial epithelial cells and mouse cells [54,55]. However, knockout of these molecules does not result in complete abrogation of poly(I:C)-induced TLR3 activation, indicating the presence of another uptake receptor. Indeed, human myeloid DCs do not express CD14 on the cell surface and an inhibitor for the scavenger receptor does not affect poly(I:C) uptake in human

myeloid DCs and epithelial cells [44]. Additionally, DEC-205 was identified as a receptor for ODNs in mouse DCs [56], but this is not the case of human DCs (M. Tatematsu and M. Matsumoto, unpublished work). Hence there must be several uptake receptors that participate in the cell entry of RNAs/DNAs in a cell type- and/or species-specific manner.

exRNA-INDUCED TLR3–TICAM-1 SIGNALLING

Following TLR3 oligomerization, TICAM-1 is recruited to the TLR3–TIR domain that activates the transcription factors, IRF-3 (IRN regulatory factor-3), NF-κB (nuclear factor κB) and AP-1, leading to the production of IFN-β and proinflammatory cytokines, as well as DC maturation [57] (Figure 2). exRNA-induced TLR3–TICAM-1-mediated signalling is classified into two categories; one that induces innate responses and the other that induces adaptive immune responses. The fibroblasts and epithelial cells that express TLR3, but not TLR7, -8 and -9, produce IFN-β and proinflammatory cytokines in response to viral dsRNA and structured RNA, which induce anti-viral states by inducing IFN-stimulated genes [26]. Host RNAs released from damaged cells could be taken up through raftlin-mediated endocytosis and activate TLR3, if they form functional structures as observed in PV RNA. Bernard et al. [42] showed that small nuclear RNAs derived from UV-damaged cells induced inflammation through activation of TLR3, but how these RNAs are delivered to endosomes and interact with TLR3 remains unknown.

Another important TLR3 signal is the induction of adaptive immune responses in myeloid DCs. TLR3 is highly expressed in the professional antigen-presenting DCs, including mouse CD8α^+ DCs and human BDCA3$^+$ DCs [58,59]. Myeloid DCs mature as a result of TLR3 activation through the expression of co-stimulatory molecules, NK-activating molecules including INAM (IRF-3-dependent NK-activating molecule) [60], and unidentified molecules involved in cross-presentation pathways, leading to the activation of NK cells and CTLs (cytotoxic T-cells) [61]. The TICAM-1–TBK1–IRF3 axis downstream of TLR3 is critical for gene induction involved in mouse DC-mediated NK/CTL activation [62,63]. In addition, mouse DCs produce the Th1-type cytokines, IFN-β and IL-12 (interleukin-12), via the TLR3–TICAM-1 pathway. This facilitates NK/CTL induction. Mouse DCs efficiently phagocytose the cell debris of virus-infected cells and mature through virus RNA-induced TLR3 activation [64].

The most intriguing finding is a link between TLR3 signals and epigenetic modifications [65]. Knockdown of TLR3 or TICAM-1 blocks the induction of human iPSCs (induced pluripotent stem cells) by retroviral reprogramming in human fibroblasts [65]. Poly(I:C)-induced TLR3 activation accelerates the development of iPSCs induced by the non-viral methods in fibroblasts. TLR3 activation leads to chromatin modification in fibroblasts by promoting genome-wide epigenetic alterations. These findings enable us to offer a new concept that RNA is an extracellular mediator that accounts for a broad range of TLR3–TICAM-1-mediated gene expression compared with other RNA-sensing receptors.

CONCLUSIONS

In plants, insects and nematodes, dsRNA-induced Dicer-mediated RNA interference is a powerful strategy for protection against viral infection [66–68]. Extracellular dsRNA is taken up into cells and systematically induces gene silencing [69,70]. In *Caenorhabditis elegans*, the membrane proteins SID-1 and SID-2 act as transporters of extracellular dsRNA, whose ability is dependent on the length of the dsRNA [71,72]. On the other hand, vertebrates have developed a wide range of anti-viral strategies, including an array of PRRs in the innate immune system, the IFN/cytokine system and the adaptive immune system. Extracellular dsRNAs are delivered to endosomal TLR3 that induce innate and adaptive anti-viral immunity. Additionally, structured RNAs with incomplete stem structures are recognized by both the dsRNA uptake receptor and TLR3, which may participate in the virus- or host-derived RNA-induced immune responses during infections or inflammation. The identification of the uptake receptor for dsRNAs and structured RNAs in human cells and also isolation of endogenous or exogenous TLR3-activating RNA molecules are important for improving our understanding of TLR3-mediated immunity.

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NMR STRUCTURE NOTE

The N-terminal domain of TIR domain-containing adaptor molecule-1, TICAM-1

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Biological context

Toll-like receptors (TLRs) are a family of single-span transmembrane proteins that evoke innate immunity in response to microbial stimuli such as bacterial lipids and non-self nucleic acids (reviewed in Botos et al. 2011 and Kang and Lee 2011). After ligand binding, TLRs oligomerize and undergo conformational changes that induce oligomerization of the cytosolic Toll/interleukin-1 receptor (TIR) domains. This presents a scaffold for the recruitment of downstream TIR domain-containing adaptor molecules.

There are five TIR domain-containing adaptor molecules; myeloid differentiation primary response gene 88 (MyD88), TIR domain-containing adaptor protein (TIRAP) [also known as MyD88 adaptor like (Mal)], TIR domain-containing adaptor molecule-1 (TICAM-1) [also known as TIR domain-containing adaptor-inducing IFN-β (TRIF)], TIR domain-containing adaptor molecule-2 (TICAM-2) [also known as TRIF-related adaptor molecule (TRAM)], and sterile α and huntingtin-elongation-A subunit-TOR armadillo motifs (SARM). Downstream signaling from TLRs is mediated by the association of the TIR domains between receptor and adaptor, and adaptor and adaptor molecules.

TICAM-1 is a signaling adaptor for TLR3 and TLR4 that eventually activates the transcription factors, interferon regulatory factor-3 (IRF-3), NF-κB and AP-1, leading to the induction of type I interferons and inflammatory cytokines (Seya et al. 2005). TLR3 recognizes double-stranded RNA and directly interacts with the TICAM-1 TIR domain via the cytosolic TLR3 TIR domain. TLR4 recognizes lipopolysaccharides together with a cofactor molecule MD2, and interacts with Mal and the TICAM-2 TIR domain via the cytosolic TLR4 TIR domain. Mal and TICAM-2 act as membrane sorting adaptors that interact with MyD88 and TICAM-1, respectively, via TIR domains. In response to TLR stimulation, TICAM-1 alters its distribution profile in the cytosol from diffuse to a speckle-like structure that is indispensable to downstream signaling (Matsumoto et al. 2003; Funami et al. 2007). TICAM-1 consists of an N-terminal domain (NTD), a flexible region that harbors a binding site for tumor necrosis factor receptor-associated factor (TRAF) proteins, a TIR domain, and a C-terminal region that includes the receptor interacting protein 1 (RIP1) binding motif (RHIM). The TIR-domain of TICAM-1 is associated with the TIR domains of TLR3 and TICAM-2, a TLR4-bridging adaptor molecule. The TICAM-1 TIR domain is also involved in TICAM-1 homo-oligomerization (Funami et al. 2008). A TICAM-1 TIR domain mutant (Pro434 substituted to His), defective in homo-oligomerization and diffusively localized in the cytosol, abrogates NF-κB and IRF-3 activation, but retains heterotypic TIR–TIR interaction with TLR3 and TICAM-2 TIRs. Recently, we have determined the structures of the TICAM-1 and TICAM-2 TIR domain mutants (Enokizono et al. 2013). In

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combination with the structural data and yeast two-hybrid experiments using the wild types and several mutants of TICAM-1 and TICAM-2 TIR domain, the interaction site between TICAM-1 and TICAM-2 TIRs was identified.

The TICAM-1 mutant lacking NTD was reported to form a speckle-like signalsome in the cytosol without stimulation and to show interferon-β promoter activity higher than that of the wild-type TICAM-1 (Tatematsu et al. 2010), indicating that NTD interacts with TICAM-1 TIR in an autoinhibitory manner. Here, we report the NMR structure of TICAM-1 NTD and propose an interaction mode between TICAM-1 TIR and NTD.

Methods and results

Sample preparation

TICAM-1 NTD (residue range 1–156) was cloned into the pGEX-6p (GE Healthcare) plasmid. A $^{13}C/^{15}N$ labeled protein was prepared by culturing *E. coli* BL21 cells in stable isotope-labeled C.H.L. medium (Chlorella Industry). Protein expression was induced by the addition of isopropyl-1-thio-β-galactopyranoside to a final concentration of 0.01 mM. After induction, the cells were cultured at 25 °C overnight and then lysed by sonication. The GST-fused protein was purified using a glutathione-Sepharose 4B column (GE Healthcare), and GST was excised from the protein with PreScission protease (GE Healthcare). The protein was further purified by size exclusion chromatography using a Superdex 75 gel filtration column (GE Healthcare). Finally, the protein was concentrated using a Vivaspin 2–5 K ultra filtration system (GE Healthcare).

NMR assignment and structure calculation

NMR experiments were carried out at 25 °C on Varian UNITY INOVA 800 and 600 spectrometers. Measurements for structural analysis were made using a 0.75 mM protein sample resolved in 50 mM Na-phosphate buffer (pH 6.5), containing 3 mM DTT and 1 mM sodium azide. Three dimensional amide-proton-detected spectra; HN(CO)CA, HNCA, CBCA(CO)NH, HNCACB, HBHA(CO)NH, HN(CA)HA, and C(CO)NH, were obtained with a non-uniform sampling schedule method and processed using the mmrtk program (Mobli et al. 2007). [$^{1}H–^{15}N$] HSQC, [$^{1}H–^{13}C$] HSQC, HC(C)H–TOCSY, ^{13}C-edited NOESY–HSQC and ^{15}N-edited NOESY–HSQC spectra, obtained using a normal sampling schedule, were processed using the NMRpipe program (Delaglio et al. 1995). All spectral analyses were performed with the help of the Sparky program (Goddard and Kneller 1997). The ^{1}H, ^{13}C, and ^{15}N chemical shifts were referenced to DSS in accordance with IUPAC recommendations. The [$^{1}H–^{15}N$] HSQC spectrum of TICAM-1 NTD was well dispersed (Fig. 1), and all the observed $^{1}H/^{15}N$ and $^{1}H/^{13}C$ resonances were assigned except for the side chain $^{1}H/^{15}N$ resonances of Lys and Arg residues. TICAM-1 NTD contains four Cys residues (C^{3}, C^{37}, C^{109}, and C^{144}). The chemical shifts of the β-carbons of these Cys residues indicate that all of these Cys residues exist in reduced state.

Interproton distance restraints for structural calculations were obtained from ^{13}C-edited NOESY–HSQC and ^{15}N-edited NOESY–HSQC spectra using a mixing time of 75 ms. The restraints for backbone ϕ and ψ torsion angles were derived from the chemical shifts of backbone atoms using the TALOS+ program (Shen et al. 2009). The structure determination and NOE assignment were carried out using the

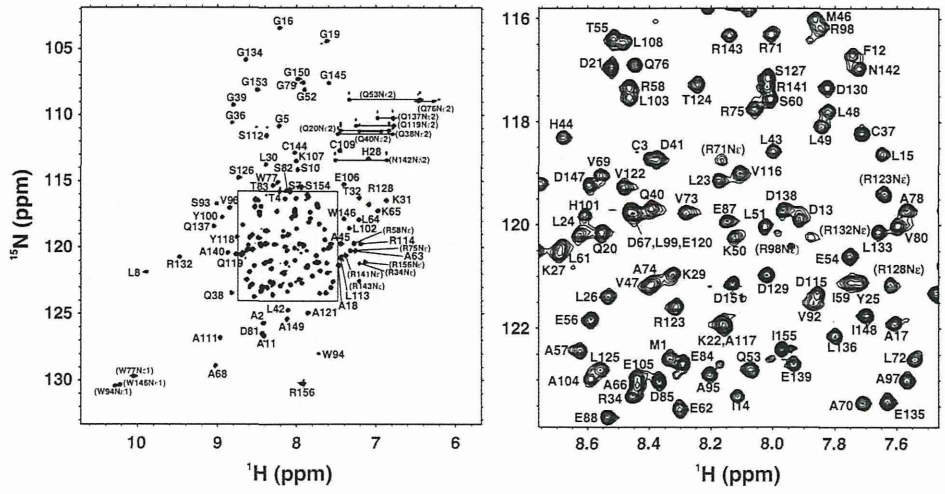

Fig. 1 [$^{1}H–^{15}N$] HSQC spectrum of the TICAM-1 NTD with resonance assignments. The *boxed region* (in *left*) is expanded on the *right*

Table 1 Structural statistics for the TICAM-1 NTD

NOE distance constraints	
Total	3,828
Short range ($\|i - j\| \leq 1$)	1,952
Medium range ($1 < \|i - j\| < 5$)	976
Long range ($\|i - j\| \geq 5$)	900
Number of violations	
Distance > 0.3 Å	3
Angle > 5°	0
Structural coordinates rmsd (Å) (residue range 5–81, 88–150)	
Backbone atoms	0.33
All heavy atoms	0.70
Ramachandran plots (%)	
Most favored regions	78.4
Additionally allowed regions	21.0
Generously allowed regions	0.6
Disallowed region	0.0

CYANA 2.1 software package (Güntert 2004). As an input for the final structural calculation of TICAM-1 NTD, a total of 3,823 distance and 231 dihedral angle restraints were used (Table 1). At each stage, 100 structures were calculated using 30,000 steps of simulated annealing, and a final ensemble of 20 structures was selected based on CYANA target function values. The determined structures were validated by CYANA macros including distance and angle violation and Ramachandran plots. The atomic coordinates and NMR data have been deposited in the Protein Data Bank (PDB code: 2M63) and BMRB (BMRB ID: 19106).

Discussion and conclusions

The solution structure of TICAM-1 NTD

The three-dimensional structure of TICAM-1 NTD was determined using standard hetero-nuclear multidimensional

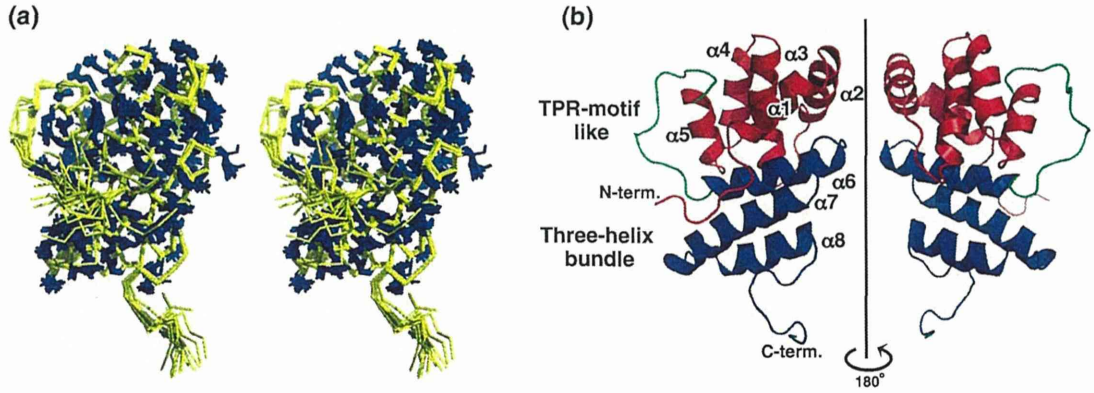

Fig. 2 Solution structure of TICAM-1 NTD. **a** Overlay of the ensemble of 20 final energy-minimized CYANA structures in stereo. Main chain and converged side chains were colored in *yellow* and *blue*, respectively. **b** Ribbon diagrams of the lowest energy structure. TPR-motif like domain, long-loop region and three-helix bundle domain were colored in *red*, *green* and *blue*. Structures were drawn using PyMOL (http://www.pymol.org/)

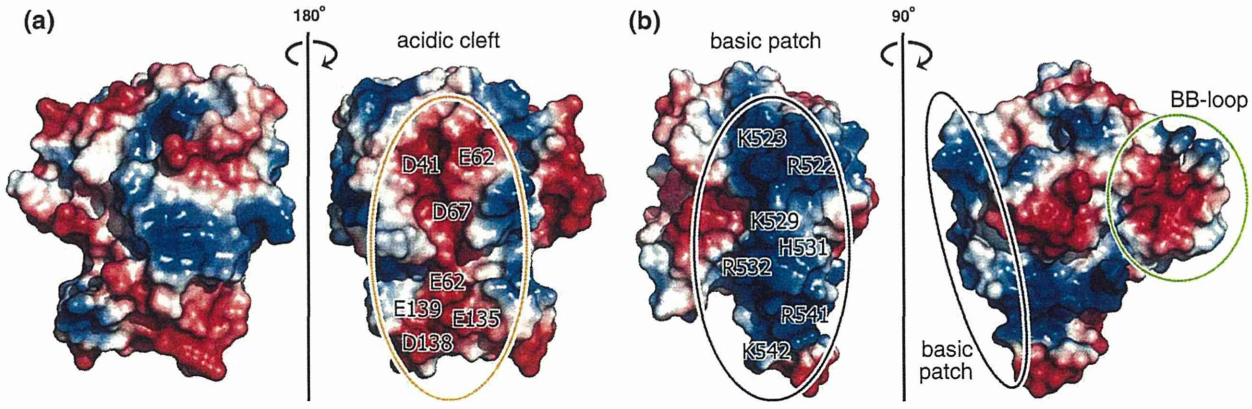

Fig. 3 Electrostatic surface potential mapped on the molecular surface of **a** TICAM-1 NTD (PDB ID: 2M63) and **b** TICAM-1 TIR (PDB ID: 2M1X). Positive and negative charge densities are colored *blue* and *red*, respectively. Surface mapping was drawn using PyMOL with APBS tools

NMR methods. TICAM-1 NTD formed a single, compact domain comprised of eight α-helices (α1: 9–18, α2: 20–31, α3: 38–51, α4: 54–64, α5: 68–76, α6: 92–106, α7: 111–128, and α8: 133–144) and a long loop region between α5 and α6 helices (Fig. 2). In this loop, the region between Ser82 and Glu87 is located that shows low convergence of backbone structure because these residues have no long range NOEs. The overall arrangement of the α-helices in TICAM-1 NTD was found to be novel after an homology search of the previously determined structures using the DALI server (Holm et al. 2008). TICAM-1 NTD can be divided into two segments, a TPR (tetratricopeptide repeat) motif-like region (α1–α5) and a three-helix bundle region (α6–α8). The TPR motif-like region has two sequentially adjacent anti-parallel α-helical pairs (α2–α3 and α4–α5), as is observed in typical TPR proteins. The TPR proteins mediate the protein–protein interactions or inter-domain assembly of multiple domain proteins. The TPR proteins generally present tandem arrays of 3–16 motifs, which form a right-handed super-helical structure and create an amphipathic groove for target recognition. However, the TPR-motif of TICAM-1 NTD does not have sufficient repeats to create a super-helical structure, and moreover, the α1-helix lies on the amphipathic groove (Fig. 2b).

TICAM-1 TIR domain binding site

Recent studies have shown that TICAM-1 NTD is an autorepression domain that directly interacts with the TICAM-1 TIR domain, leading to attenuation of the TIR–TIR interaction (Tatematsu et al. 2010). In a previous paper (Enokizono et al. 2013), we presented the TICAM-1 TIR domain structure and identified two distinct interaction sites required for homotypic and heterotypic TIR oligomerization. A hydrophobic patch that includes the BB loop was important in mediating the homotypic interaction of TICAM-1 TIR, whereas a basic patch on the αE- and αE′-helices was essential for heterotypic interaction with TICAM-2 TIR (Fig. 3b left). An acidic cleft stretched over the TPR motif-like region and three-helix bundle region (Fig. 3a right) that could interact with the basic patch of TICAM-1 TIR domain (Fig. 3b left) was found on the TICAM-1 NTD surface. This suggests the possibility that TICAM-1 NTD blocks the binding between TICAM-1 TIR and TICAM-2 TIR, thus regulating TICAM-1-mediated TLR4 signaling in an autoinhibitory manner. Further studies are required to confirm this hypothesis.

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Myeloid-Derived Suppressor Cells Confer Tumor-Suppressive Functions on Natural Killer Cells via Polyinosinic:Polycytidylic Acid Treatment in Mouse Tumor Models

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Key Words
Myeloid-derived suppressor cells · Mitochondrial antiviral signaling protein · Tumor immunotherapy · Double-stranded RNA

Abstract
Polyinosinic:polycytidylic acid (poly I:C), a synthetic double-stranded RNA, acts on myeloid cells and induces potent antitumor immune responses including natural killer (NK) cell activation. Myeloid-derived suppressor cells (MDSCs) systemically exist in tumor-bearing hosts and have strong immunosuppressive activity against antitumor effector cells, thereby dampening the efficacy of cancer immunotherapy. Here we tested what happened in MDSCs in poly I:C-treated mice. NK-sensitive syngenic tumor (B16)-bearing C57BL/6 mice were employed for this study. Intraperitoneal poly I:C treatment induced MDSC activation, driving CD69 expression and interferon (IFN)-γ production in NK cells. IFN-γ directly inhibited proliferation of B16 cells. This NK cell priming led to growth retardation of B16 tumors, although no direct tumoricidal activity was induced in NK cells. Mechanistic analysis using KO mice and function-blocking monclonal antibody revealed that MDSCs produced IFN-α via the mitochondrial antiviral signaling protein (MAVS) pathway after in vivo administration of poly I:C, and activated NK cells through the IFNAR pathway. MDSC-mediated NK cell priming was reconstituted by IFN-α in a coculture system. Either the MAVS or IFNAR signaling pathway was required for activation of MDSCs that led to growth retardation of B16 tumor in vivo. The results infer that MDSC is a target of poly I:C to prime NK cells, which exert antitumor activity to NK-sensitive tumor cells.

Introduction

The innate sensing of microbial molecular patterns results in the modulation of the cellular immune system [1–3]. This innate-adaptive linkage closely associates with suppression of infection and tumorigenesis. Many reports showed that polyinosinic:polycytidylic acid (poly I:C), a synthetic pattern of double-stranded RNA, has potent stimulatory effects on immune responses to viral infection and cancer [4–8]. Poly I:C is an agonist for pattern-recognition receptors (PRRs), Toll-like receptor 3 (TLR3) and melanoma differentiation-associated protein 5 (MDA5), which transduce signals to the adaptor molecules TICAM-1 (also known as TRIF) and mitochondrial antiviral signaling protein (MAVS; IPS-1, Cardif, VISA) [9–12]. They differentially modulate the functions of myeloid dendritic cells (DCs) and macrophages, including cytokine/IFN production and expression of surface molecules that drive effector cell activation.

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