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FIGURE LEGENDS

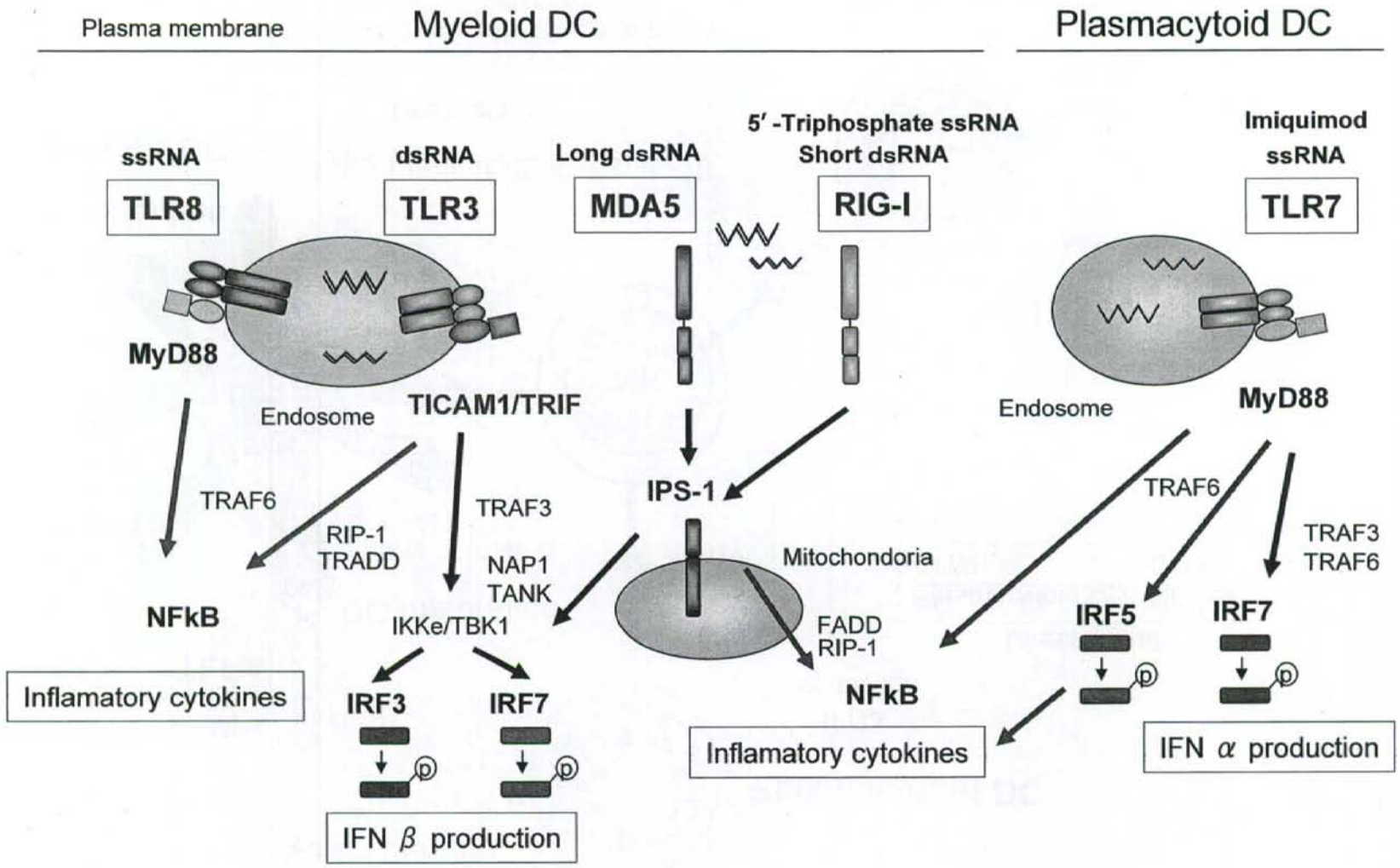
Fig. (1). How DCs sense virus-derived RNA.
Myeloid DCs and plasmacytoid DCs use different systems for defense against virus-derived RNA. Plasmacytoid DCs express TLR7 in their endosomes and produce IFNa through IRF7 activation after recognition of virus-derived ssRNA. Myeloid DCs have MDA5 and RIG-I in the cytosol and TLR3 and TLR8 in the endosomes. MDA5 and RIG-I detect virus-derived RNA (MDA5: long dsRNA, RIG-I: short dsRNA and 5'-triphosphate ssRNA) in the site where non-self RNA is produced in virus-infected cells. Extrinsic dsRNA is recognized by TLR3 in the endosomes. The MDA5/RIG-I and TLR3 signal pathways lead to activation of IRF3 and IRF7, resulting in IFNb production. While TLR8 can participate in innate immunity by detecting extrinsic ssRNA, the role of TLR8 in myeloid DC is not determined in actual RNA virus infection.

Fig. (2). Molecules that regulate the reciprocal interaction between sensor cells and NK cells.
For full activation, NK cells have to be supported by sensor cells (myeloid DCs, plasmacytoid DCs, macrophages and monocytes, in this figure) that recognize pathogen-associated molecular patterns (PAMPs). In general, NK cells and sensor cells are reciprocally activated by soluble signals and cell/cell contact. Since the tropism of the pathogen varies, the main NK activating players are determined by which sensor cells are attacked by the pathogens and induce innate signals for NK activation.

Fig. (3). HCV-infected apoptotic cells, but not HCV particles, regulate mDC function to activate NK cells and T cells.
The HCV JFH1 strain does not directly stimulate mDCs or pDCs. In this case, pDCs and mDCs do not directly respond to the RNA virus. Our recent finding is that dsRNA in HCV-infected apoptotic vesicles is an immune-stimulator for mDCs that triggers the innate system for the activating T cell (Th1 dominant) and NK cells. Cell/cell contact between mDCs and NK cells is indispensable to increased NK cell cytotoxicity.

Fig. (4). The TLR3/TICAM1 signal leads to expression of unknown molecules on mDC, which activate NK cell via cell/cell contact.
Among the three poly I:C receptors (TLR3, RIG-I and MDA5), the TLR3/TICAM1 signal plays an important role in regulating mDC capacity to activate NK cells. This

mDC-mediated NK activation is dependent on the direct interaction of mDC and NK cells. Besides IL-15, there must be other key molecules that are induced on mDC by the TLR3/TICAM1 signal and which regulate NK activation. NK activity is also determined by induction of type I IFN through MDA5. Although IL-12 is produced mainly by the TICAM1 signal, IL-12 is not a functional entity that can activate NK cells by poly I:C-stimulated mDCs.


Plasma membrane
Myeloid DC
Plasmacytoid DC
ssRNA
TLR8
dsRNA
TLR3
Long dsRNA
MDA5
5′ -Triphosphate ssRNA
Short dsRNA
RIG-I
Imiquimod
ssRNA
TLR7
MyD88
Endosome
TICAM1/TRIF
Endosome
MyD88
TRAF6
RIP-1
TRADD
TRAF3
NAP1
TANK
IKKe/TBK1
IPS-1
Mitochondoria
FADD
RIP-1
TRAF6
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NFkB
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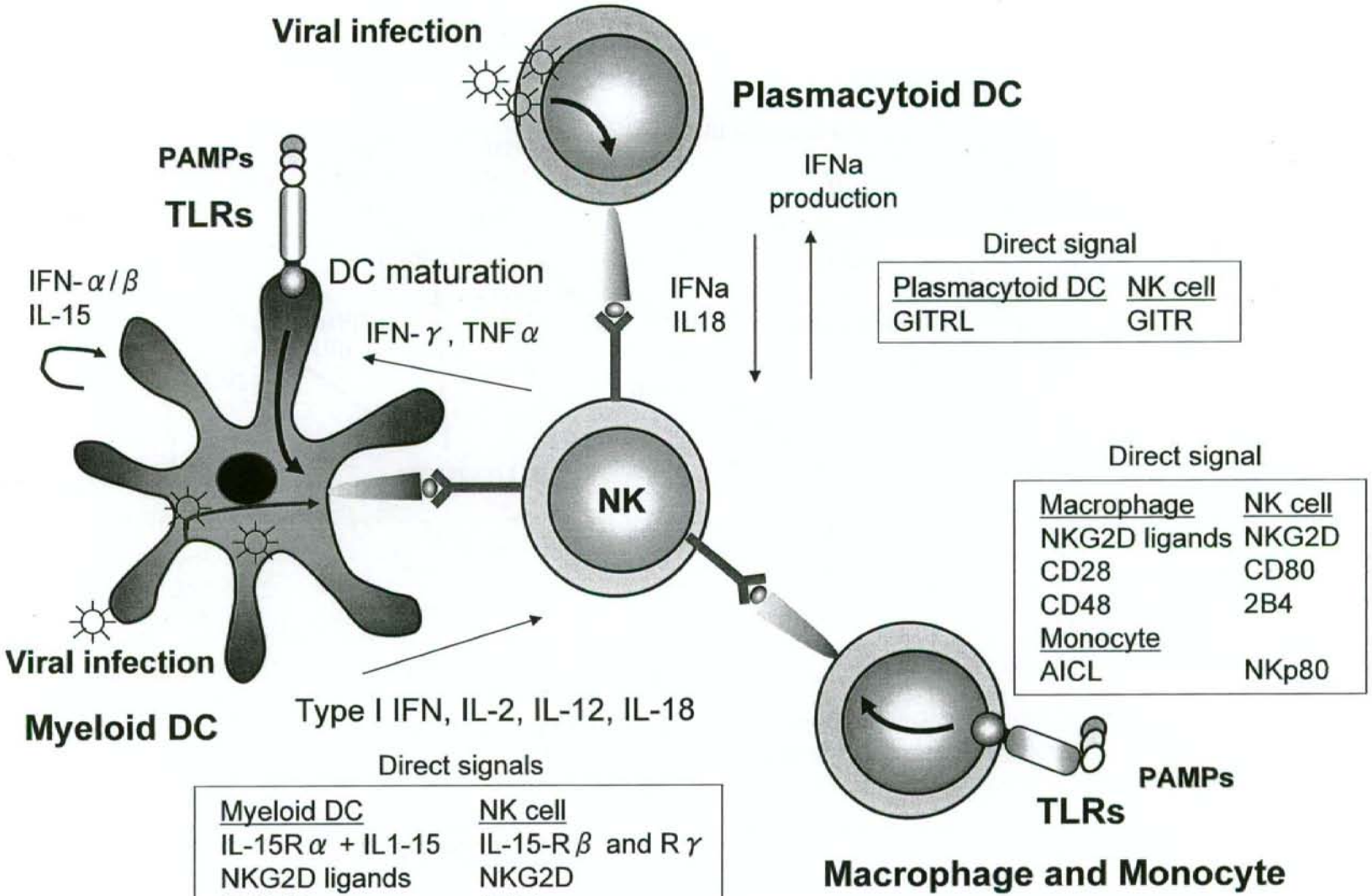
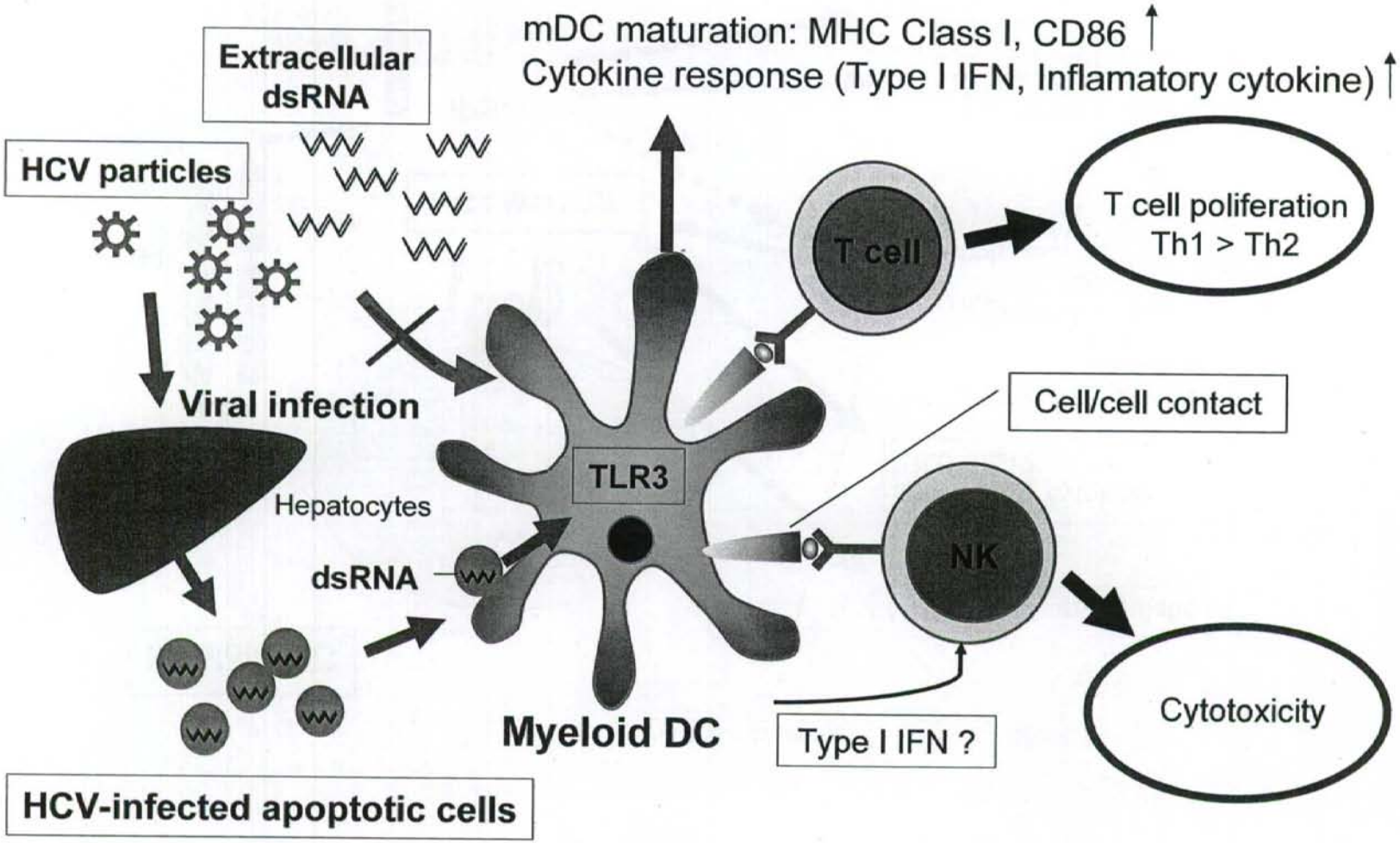
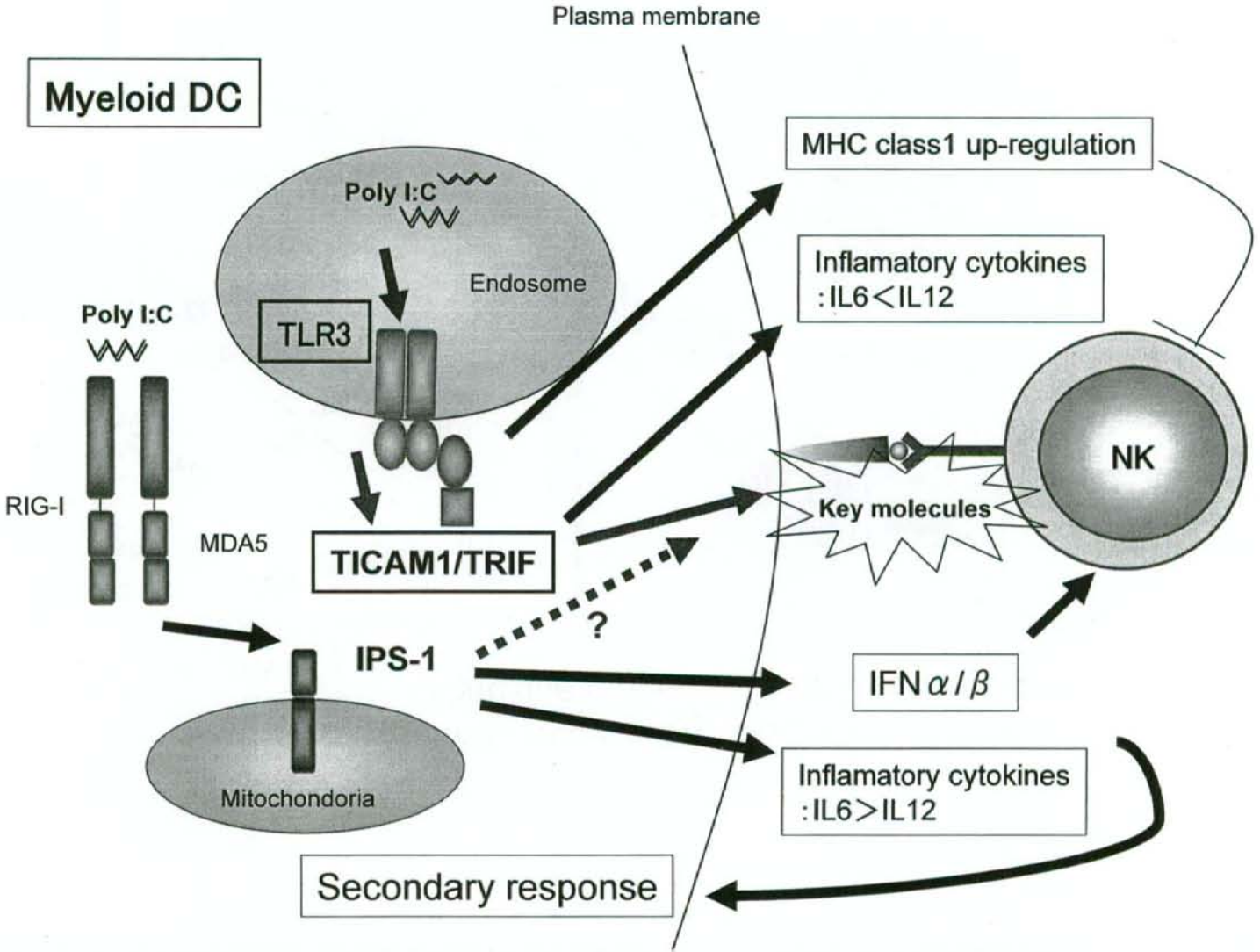
IRF3
IRF7
IFN β production
NFkB
Inflamatory cytokines
IRF5
IRF7
IFN α production


Viral infection
Plasmacytoid DC
PAMPs
TLRs
IFNa
production
Direct signal
Plasmacytoid DC NK cell
GITRL GITR
DC maturation
IFN-α/β
IL-15
IFN-γ, TNF α
IFNa
IL18
NK
Direct signal
Macrophage NK cell
NKG2D ligands NKG2D
CD28 CD80
CD48 2B4
Monocyte
AICL NKp80
Viral infection
Myeloid DC
Type I IFN, IL-2, IL-12, IL-18
PAMPs
TLRs
Macrophage and Monocyte
Direct signals
Myeloid DC NK cell
IL-15Rα + IL1-15 IL-15-Rβ and Rγ
NKG2D ligands NKG2D
ICAM1 LFA1
CLRB CD161B
Jagged2 Notch


mDC maturation: MHC Class I, CD86 ↑
Cytokine response (Type I IFN, Inflamatory cytokine) ↑
Extracellular dsRNA
HCV particles
Viral infection
Hepatocytes
dsRNA
HCV-infected apoptotic cells
TLR3
Myeloid DC
T cell
T cell poliferation Th1 > Th2
Cell/cell contact
NK
Type I IFN ?
Cytotoxicity


Plasma membrane
Myeloid DC
MHC class1 up-regulation
Poly I:C
Endosome
TLR3
Inflamatory cytokines
:IL6<IL12
Poly I:C
NK
RIG-I
MDA5
Key molecules
TICAM1/TRIF
?
IPS-1
IFN α / β
Inflamatory cytokines
:IL6>IL12
Mitochondoria
Secondary response

Cancer Immunol Immunother
DOI 10.1007/s00262-008-0652-9

REVIEW

The extrinsic RNA-sensing pathway for adjuvant immunotherapy of cancer

Tsukasa Seya · Misako Matsumoto

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Abstract Infection with RNA viruses presents a typical pattern of virus products, double-stranded RNA (dsRNA), and induces the maturation of antigen-presenting dendritic cell (mDC). There are several dsRNA sensors that are differentially distributed on the cell membrane and in the cytoplasm and are variably expressed depending on the cell type. Among these sensors, TLR3 links to the adaptor TICAM-1 (TRIF), which is characterized by its unique multipronged signaling cascades for cytokine/chemokine production, apoptosis and autophagy in both immune and tumor cells. In the context of mDC maturation, various cellular events are further induced in response to dsRNA; these include cross-priming followed by CD8+ CTL induction, NK activation and proliferation of CD4+ T cells including Th1, Th2, Treg and Th17 cells. In this review, we focus on the potential role of dsRNA in modulating the inflammatory milieu around mDCs and tumor-associated antigens to drive specific cellular effectors against the tumor.

Keywords Immunotherapy for cancer · RNA adjuvant · Toll-like receptor · TICAM-1 (TRIF) · Dendritic cells · Cellular effectors

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Introduction

Tumor progression often occurs during inflammation because cell growth is an event that is closely connected to both extrinsic and intrinsic inflammatory stimulation [1]. Many biological mediators such as cytokines and chemokines are involved in immune cell recruitment, which accelerates tumor development in an inflammatory milieu [2]. Immune-related cells are incorporated into the tumor matrix and evoke complicated immune responses against the tumor through cell–cell interactions. Ultimately, the antigen (Ag)-presenting cells (APC) mature as a result of the inflammatory stimuli and tumor-associated antigens (TAAs) and flow out to the regional lymph nodes where TAAs are presented to lymphocytes [3]. However, tumor remission does not occur frequently despite TAA presentation by APC [3, 4]. In contrast, most other infections facilitate myeloid dendritic cell (mDC) maturation [5] and provoke a robust immune response that contributes to pathogen eradication. If PRRs fail to be activated due to the lack of appropriate microbial patterns in APC of cancer patients even in the presence of TAAs, no effectors are generated for tumor targeting, thereby neither immune edition nor surveillance occurring against tumor.

Double-stranded (ds) RNA is a product of virus replication. A variety of RNA and DNA viruses generate replication-mediated dsRNA, polyU/UC or stem-loop structures [6], which serve as ligands for pattern-recognition receptors (PRRs). TLR3 [7], TLR22 [8], RIG-I/MDA5 [9], PKR [10], NALP3 [11, 12] and Dicer in the RNAi system [13] along with as yet unidentified receptors are believed to serve as PRRs for dsRNA sensing (Fig. 1a). These PRRs induce intracellular signaling cascades that regulate cell growth, differentiation, apoptosis and immune activation [6, 14]. Ultimately, dsRNA and its synthetic analog polyI:C

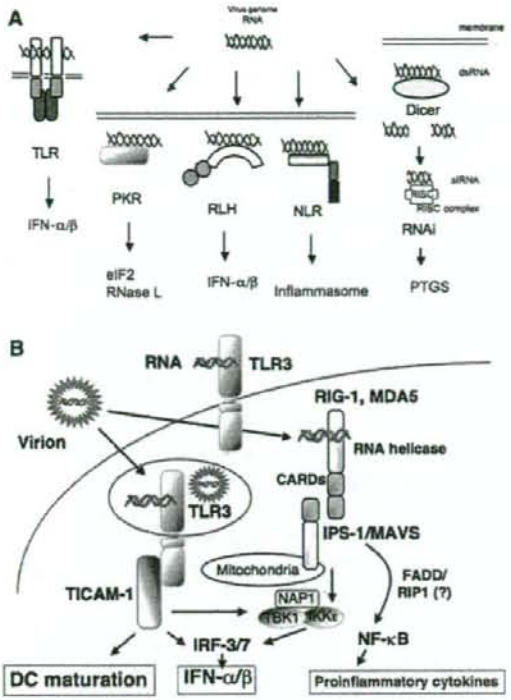

Fig. 1 dsRNA-sensing systems. **a** Double-stranded (ds)RNA are primarily generated during virus replication. Major dsRNA sensors in human cells are indicated. Dicer and RNA-recognizing helicases work in invertebrates as antiviral receptors, but in humans no evidence of these receptors for antiviral action has been proposed. How dsRNA selects a variety of RNA pattern sensors remains largely unknown. PTGS, post-transcriptional gene silencing. **b** TLR3 is mainly localized in the endosome of limited cell types, while RLH (RIG-I and MDA5) are ubiquitously distributed in the cytoplasm. Adaptor molecules, TICAM-1 and MAVS, are localized in the cytoplasm. Upon stimulation, TLR3 recruits TICAM-1 near the endosomal membrane, while MAVS recruits RLH on the mitochondrial membrane. The known outputs of TLR3 and RLH are indicated by *red*

exert a wide range of biological activities and can elicit immune responses. Since dsRNA-sensing PRRs are distributed across a variety of host cells in different combinations [6, 15], systemic inflammation occurs in various modes depending on the receptors and cell types involved in viral infection, virus vaccine inoculation or dsRNA administration for RNA therapy. An inflammatory environment promotes tumor growth and priming of dendritic cells. Many sterile and infectious RNAs induce inflammation.

The signaling pathways of PRRs are linked via adaptor proteins (Fig. 1b). The intra-cytoplasmic RNA sensors, RIG-I and MDA5, interact with MAVS (Cardif/IPS-1/VISA) on the outer membrane of mitochondria [16], and TLR3 resides in the endosome and interacts with TICAM-1 (TRIF) [17]. The signal selection systems of other dsRNA sensors are relatively less defined. Typically, stimulation of the TICAM-1 and MAVS pathways induces type I interferons (IFN) [18]. This is a reflection of the fact that the signaling cascades of both pathways converge upon the complex of the virus-activated kinase (VAK), i.e., NAP1/SINTBAD-IKKε/TBK1 [18, 19] (Fig. 2a). Other cellular responses, autophagy [20], proliferation [21] and apoptosis [22], are induced in cells stimulated with dsRNA (Fig. 2a). Study of the molecular mechanism of these responses is currently underway.

In mDCs, a variety of cellular effectors are driven in response to dsRNA. CD4 Th1, CD8 CTL, NK cells, regulatory T cells (Treg), and Th17 cells are activated/proliferated through dsRNA-stimulated mDCs [15]. Some inflammatory cytokines and chemokines, as well as IFN-inducible gene products are also up-regulated in mDCs. These effectors appear to be independently induced in a situation-dependent manner. However, the molecular mechanisms whereby these variable effectors are differentially induced by mDCs are unknown. We have determined that the TICAM-1 pathway in mDCs is involved in inducing all these effector cell types (Fig. 2b).

In this review, we focus on the TICAM-1 pathway in which cellular effectors are induced by mDCs. We also discuss the involvement of the TICAM-1 pathway in cancer progression and the therapeutic potential of TICAM-1 in antitumor immunotherapy.

TLR3 agonists in cancer immunotherapy

PolyI:C is a representative agonist for human and mouse TLR3 [23]. This compound is believed to be an analog of viral double-stranded RNA (dsRNA) and is a strong inducer of type I IFN in both humans and mice [24]. Initially, polyI:C was regarded as a PKR activator [25]. Later, it was determined that this compound is not only a TLR3 agonist, but also a stimulator of the cytoplasmic RNA sensor, MDA5 [26]. PolyI:C also activates RIG-I [26], but other viral RNA patterns, 5′-triphosphate [27, 28] and polyU/UC [29] may be natural ligands for RIG-I. Earlier, it was reported that polyI:C, which is capable of activating various PRRs, causes endotoxin-like cytokine stormsl; therefore, this compound was deemed to be too toxic for application in clinical therapy [30].

mDCs mature into APCs that drive cellular effectors (Fig. 3). TLR3 resides in the endosome of mDCs [17], senses dsRNA in the endosome, and relays signals to the TICAM-1 pathway, thereby leading to maturation of mDCs [6]. Thus, endosomal stimulation of TLR3 by ligands links to activation of mDCs (Fig. 1b). Certain dsRNA derivatives preferentially activate TLR3 rather than RLH receptors

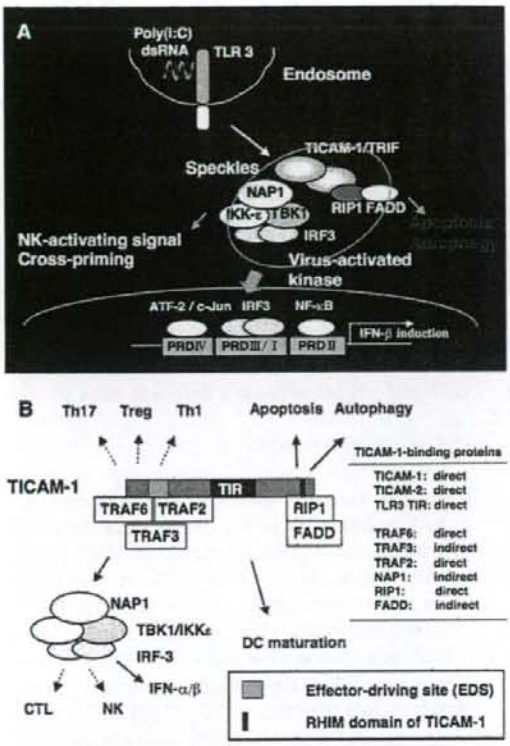

Fig. 2 An outline of the TICAM-1 pathway. **a** In human cells, TICAM-1 once detached from TLR3 serves as a signaling platform to induce apoptosis, autophagy, NK activation and cross-priming. TICAM-1 undergoes some modification secondary to complex formation with TLR3, forms multimer, and dissociated from TLR3 with unknown mechanism. The pathways for NK activation, CTL induction and autophagy are only partially identified, although the pathway for apoptosis is getting clarified. Although epithelial cells in bronchi, bile-duct and intestine express TLR3 on their surface membranes, it is undetermined whether surface-expressed TLR3 retains the cellular responses. **b** The N-terminal 'Effector-driving site (EDS)' recruits appropriate signal-transmitting molecules and matures mDCs leading to induction of effector cells, including NK and CTL. The C-terminal RHIM domain participates in signal transmission for apoptosis and autophagy. TICAM-1-binding proteins, either direct or indirect, are summarized in the inset table

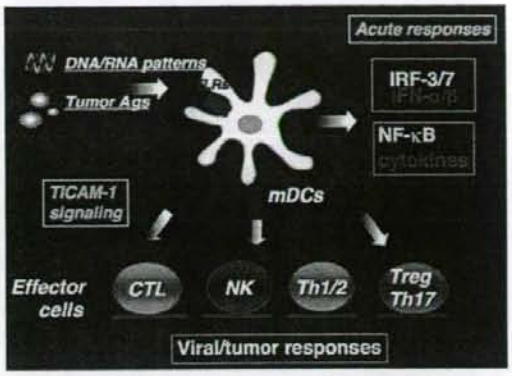

Fig. 3 Various effectors driven by the TICAM-1 pathway in mDCs. The effectors can be induced through the TICAM-1 pathway in mDCs are delineated in this figure. In an early phase of infection, cytokines and IFNs are released in response to microbial patterns. Later, the cellular effectors are induced secondary to activation of the TICAM-1 pathway in mDCs

when they are targeted to the endosome [6]. Synthetic or viral replication-induced RNA products with the stem or stem-loop structures possess mild TLR3-agonistic activity and have no toxic effect on mice. These modified RNA duplex signatures are potential TLR3 stimulants.

Although the natural ligands of TLR3 remain unknown, TLR3 recognizes RNA duplex. To date, it has been shown that polyI:C and the duplex signatures of RNA from many viruses and other synthetic RNAs can be recognized by TLR3. DOTAP and other lipofection agents can deliver RNA to the endosome where TLR3 is localized in mDCs [6]. TLR3 links to the adaptor TICAM-1 to induce IFN-β signaling. Whether or not TLR3 links to MyD88 in addition to TICAM-1 in mDCs remains unknown. However, based on the results from knock-out (KO) mice analyses, the contribution of the MyD88 pathway to the functioning of mDCs is minimal, if any [31].

TICAM-1 signaling

TICAM-1 is the largest of the four TLR adaptors identified so far [32]. It serves as a platform for the assembly of the TRAF family [33, 34] and TANK family [18, 19]. The N-terminal region of TICAM-1 [named Effector-driving site (EDS)] participates in the molecular recruitment (Fig. 2b). In contrast, RIP1 [35] and FADD [36] are recruited to its C-terminal region (Fig. 2b). A variety of cellular outputs were then developed [34, 37]. IFN-α/β, proinflammatory cytokines, ROS and K+ are induced in mDCs. Autophagy and apoptosis are evoked in cells other than mDCs. TICAM-1 modification and translocation lead to the formation of TICAM-1 homo-multimers in mDCs, which activate signal pathways leading to induction of cellular effectors, CTL, NK and CD4+ T cells [15]. The IFN-α/β-inducing pathway of TICAM-1 has been well characterized. Interferon regulatory factor (IRF)-3 and -7 are activated by virus-activated kinase (VAK) [38]. A similar pathway induces IL-1β, IL-6, TNF-α and IL-12p40 [38]. However, the pathways by which ROS are induced remains unknown. Recent reports suggest that LPS, a ligand that activates the TLR4-TICAM-1 pathway [39], induces the activation of

the inflammasome which may interfere with autophagy. This leads to incremental production of IL-1β as well as ROS [40]. Thus, entire pathways led by TICAM-1 remain to be characterized but the pathways appear to coordinately diverge to induce different effectors.

Two PRRs link the TICAM-1 adaptor in humans and mice. TLR3 directly couples with TICAM-1 [41, 42], whereas TLR4 recruits the TICAM-2 (TRAM)-TICAM-1 complex in human and mouse cells [39]. Once dsRNA is provided exogenously, it is taken up into the endosome where TLR3 is expressed [43]. When TLR3 is stimulated, TICAM-1 is recruited to the cytoplasmic TIR domain of TLRs and then dissociated from the receptor, leading to multimer formation [43, 44]. Multimeric TICAM-1 is capable of assembling TRAF family proteins (particularly TRAF2, 6 and 3) in the N-terminal region of TICAM-1 [33]. This ubiquitin E3 ligase complex binds VAK, consisting of NAP1 (or other TANK family proteins), IKKε and/or TBK1. VAK in turn activates IRF-3 and IRF-7 in the cytoplasm [38]. The phosphorylated IRFs translocate to the nucleus to activate the IFN-α/β promoters. The MAPK pathway may be activated through the N-terminal region of TICAM-1. On the other hand, the C-terminal portion of TICAM-1 recruits RIP1, which leads to the activation of IKKα/β and NK-κB [35]. These pathways sustain the production of inflammatory cytokines and type I IFNs. Although the TICAM-1 protein is maintained at low levels in normal cells, the mechanism by which this protein is regulated remains unknown.

In contrast, MAVS, which is the adaptor molecule of RIG-I/MDA5 for signaling the presence of cytoplasmic dsRNA, also binds TRAF (3 and 6), TRADD and RIP1 in the outer mitochondrial membrane to activate VAK [45]. If this protein is cleaved at the C-terminus by the NS3/4A protease of HCV, it loses the ability to transduce signaling to VAK [46]. It also inactivated by proteolytic cleavage by caspase 1 [47].

The TICAM-1 pathway in cancer cells

Tumor cells induce autophagy via the TICAM-1 pathway [48]. PolyI:C is a compound that induces autophagy in tumor cells, and this reaction augments the activation of caspase 1 of the inflammasome that produces robust amounts of active IL-1β, IL-18 and IL-33 [49]. TICAM-1 KO cells lose the ability to undergo polyI:C-mediated inflammasome activation. This autophagy-augmenting activity is TICAM-1-dependent, and has been mapped to the N-terminal region of EDS.

Breast cancer cells undergo apoptosis upon treatment with polyI:C [50]. Intestinal epithelial cells of mice are injured upon intraperitoneal administration of polyI:C [51]. Previous studies have shown that TICAM-1-overexpressing cells induce apoptosis through a RIP/FADD/caspase 8-dependent pathway [52]. PKR may be additionally involved in dsRNA-derived apoptosis [53]. TLR3 as well as PKC-alpha plays a part in poly(I:C)-mediated tumor cell apoptosis [54]. In other reports, cell damage and apoptosis by polyI:C were not merely due to the TICAM-1 pathway, but were a consequence of the output secondary to other dsRNA-sensing pathways [22, 52–54].

Some tumor cell lines induce IL-6, IL-12p40, IL-1β, TNF-α and IL-8 in response to polyI:C. Of these, IL-12p40 induction is largely dependent on TICAM-1 [55]. Other cytokines partly depend on TICAM-1 and the MAVS pathway.

CTL and NK cell activation driven by mDCs

CTL is induced by TICAM-1

CTLs proliferate in response to Ags presented on MHC class I molecules in mDCs. Endogenous Ags, including proteins of viral origin, are presented on MHC class I molecules to induce MHC-restricted CTL in virus replication. Since dsRNA is produced along with Ag presentation in virus-infected mDCs, viral Ags are efficiently presented in a TAP-dependent manner under these circumstances, and pattern molecules, which are dsRNA molecules in this case, simultaneously stimulate mDCs. However, mDCs are not always infected with viruses and even when they are non-infected, they can present viral Ag in a TAP-independent manner [56]. In other word, when Ags and dsRNA are extrinsically taken up into mDCs, the cross-priming mechanism enables mDCs to present Ags on MHC class I molecules [56, 57]. Cross-priming is enhanced by the TICAM-1 pathway in mDCs (Fig. 2a), which efficiently induce Ag-specific CTL [58]. It is expected that similar dsRNA-mediated cross-priming occurs in mDCs that phagocytose TAA instead of viral Ags [3]. Activation of the pathway that induces CTL against TAAs may occur in mDCs and this may facilitate the regression of MHC-high tumors.

Based on increasing evidence obtained by deletion mutagenesis experiments, the N-terminal region of TICAM-1 is involved in the initiation of cross-priming in mDCs. The region contains the site required for TRAF-binding and VAK activation, and probably overlaps with EDS (Fig. 2b). However, induction of cross-priming is independent of IRF-3/7. Thus, occurrence of this event relies on the mechanism involving the molecules for VAK activation but is not dependent on the transcription factors IRF-3/7 [59].

NK cells are induced by TICAM-1

NK cell activation is reciprocally induced by dsRNA-stimulated mDCs (Fig. 4). The mDC-activated NK cells

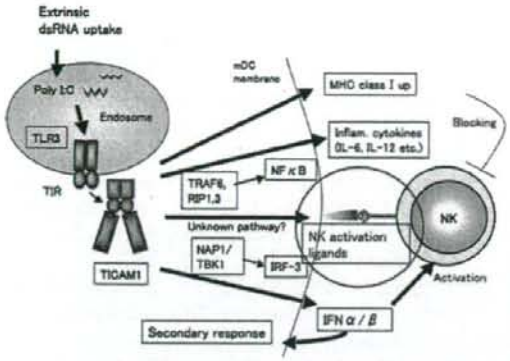

Fig. 4 Possible NK-inducing pathways against cancer. NK activation is an example of mDC output. For full activation, NK cells have to be supported by dendritic cells (myeloid DCs in this figure) that recognize pathogen-associated molecular patterns (PAMPs). In general, NK cells and dendritic cells are reciprocally activated by soluble signals and cell/cell contact. Since the tropism of the pathogen varies, the main NK activating players are determined by which sensor cells are attacked by the pathogens and induce innate signals for NK activation

effectively damage MHC-low tumor cells. The events involved in mDC-mediated NK activation upon stimulation with dsRNA remains unknown. TICAM-1 KO mDCs fail to activate NK cells and TICAM-1 KO NK cells fully restore IFN-γ induction and cytotoxic activity against NK target cells [60], suggesting that mDC TICAM-1 is essential for NK driving. Transwell experiments have revealed that cell–cell contact between mDCs and NK cells rather than with mDC-liberated mediators is crucial for mDC–NK activation [60]. The molecule responsible for NK activation must be expressed on the surface of mDCs in response to dsRNA stimuli and foster mDC–NK interaction (Fig. 4). Genechip analyses using TICAM-1 KO versus wild-type mDCs stimulated with polyI:C, have permitted the identification of several molecules as TICAM-1-dependent NK activation enhancers. NK activation followed by mDC maturation has a strong antitumor effect against MHC-low tumors. The TICAM-1 region required for NK driving in mDCs is the N-terminal region that includes the EDS of TICAM-1 (Fig. 2b). Induction of IRF-3, not IRF-7, is essential for this mDC-NK reciprocal activation [59].

Induction of Th, Treg and Th17 cells by mDCs

CD4+ Th cells

CD4+ Th cells play a pivotal role in skewing the immune responses against cancer. Th1 effector cells are critical for the maintenance of memory CD8+ T cells [61–63], while Th2 cells help B cells to produce various classes of immunoglobulins (Ig) [64, 65]. It is not completely clear as to how memory T cells are regulated by CD4+ T cells, but the importance of CD4+ T cells in the generation and expansion of CD8+ memory T cells has been reported [66]. Earlier data on CD4+ T cell functions should be interpreted cautiously since in those studies, the CD4+ Th populations frequently contained CD4+ regulatory T (Treg) and Th17 cells, and these contaminating cells acted in concert with CD4+ Th cells to modulate the development of CD8+ memory T cells. The possible roles of Treg and Th17 cells in tumor progression will be discussed later. In general, Treg cells suppress immune responses to induce immuno tolerance at tumor sites [67], while Th17 cells are evoked in conjunction with acute inflammation and are linked to smoldering inflammation around the tumor lesion to promote tumor incidence and growth [68]. The functions of CD4+ Th cells should be defined by discounting these Treg/Th17 effector functions.

The CD4+ Th cells consist of the Th1 and Th2 T cell subsets, based on their distinct cytokine secretion profiles. CD4+ Th1 cells produce cytokines IL-2 and IFN-γ. The latter is produced by Th0 (naive T) cells after IL-12 from mDCs stimulate the expression of Stat1 and subsequently that of T-bet, a master transcription factor in Th1 cells [69]. The TICAM-1 pathway in mDCs may contribute to Th1 polarization by preferentially inducing IL-12p40 [55, 60, 70]. CD4+ Th1 cells then provide cytokines for CD8+ T cells and synergistic activation of mDCs, which are essential for CD8+ T cell proliferation and function [71]. IL12p40 is a cytokine that is induced by VAK, which connects with the N- and C-terminal regions of TICAM-1 (Fig. 2b).

In contrast, some TLR ligands may promote the differentiation of CD4+ Th2 cells. IL-4 produced by basophils, eosinophils and NKT cells initiates Stat6 signaling, leading to the expression of GATA-3, which is a master transcription factor in Th2 cells [71]. Participation of TICAM-1 in Th2 polarization has been reported [72] but not confirmed by another group [73]. Several attempts have been made to establish CD4+ T cell clones from tumor-infiltrating T cells. The results indicated that most CD4+ T cell clones are Th1 effectors that secrete IFN-γ and IL-12, but not IL-4 [74].

Th17 cells

IL-17-producing T (Th17) cells are a distinct lineage within the general category of CD4+ Th cells, and secrete a unique set of cytokines, i.e., IL-17 [75, 76]. TGF-β and IL-6 produced by tumor cells, Treg cells and APCs activate the TGF-β and Stat3 signaling pathways, leading to the expression of RORγt, a critical transcription factor for Th17 cells [77]. Th17 cells were first identified as a new CD4+ T cell subset consisting of self-reactive CD4+ Th1 cells. These

cells were later associated with the pathogenesis of many autoimmune diseases [75, 76]. The role of Th17 cells in cancer is less defined than that of Th1 cells. Nonetheless, both IL-17 and IL-23 have been identified in cancer tissues [78], suggesting that Th17 cells together with proinflammatory cytokines may provide an environment favorable for cancer development or invasion. We recently showed that elevated lactic acid in cancer tissues and macrophages in response to TLR stimuli play a key role in IL-23 induction in mDCs or tumor-associated macrophages and help inducing Th17 cells in cancerous environments [79]. Thus, the induction of both IL-23 and IL12p40 by TICAM-1 may be crucial for Th17 stimulation in mDCs. Th17-mediated development of autoimmune disease is constrained by TICAM-1-dependent type I IFN production and its downstream signaling pathway [80]. However, the TICAM-1 region in mDCs that participates in Th17 development is unknown. Th17 cells might play certain roles in tumor progression.

Treg cells

CD4+ Treg cells have been identified as a small subset of the T cell population. Several subpopulations of Treg cells have been reported. Naturally occurring CD4+/CD25+ Treg cells together with other CD4+ Treg cells, including CD4+/CD25− Treg, Tγ-1 and/or Th3 cells, are involved in T cells regulation [81]. Tγ-1 cells secrete IFN-γ and IL-10, while Th3 cells secrete high levels of TGF-β, IL-4 and IL-10. Foxp3 has been shown to be a specific marker of CD4+ Treg cells in both mice and humans [82, 83]. Its expression is highly restricted to the subset of Treg cells and is correlated with immunosuppressor activity, irrespective of CD25 expression.

CD4+ Treg cells can suppress host immune responses to a great extent and induce self-tolerance. Thus, despite their protective role in autoimmune diseases, these cells have inhibitory effects on cancer immunotherapy and anti-infectious responses [84]. That is, malignant tumors tend to progress more rapidly in a Treg-dominant environment. Recent studies have shown that the proportion of CD4+/CD25+ Treg cells was elevated in the total CD4+ T cell population in several different human cancers, including lung, breast and ovarian tumors [85, 86]. Ag-specific CD4+ Treg cells are situated at tumor sites, and these cells suppress the proliferation of naïve CD4+ Th cells upon activation by tumor-specific Ags [87]. TLR8 regulates CD4+ Treg function by sensing RNA in Treg cells: adoptive transfer of TLR8 ligand-stimulated Treg cells into tumor-bearing mice enhanced antitumor immunity [88]. Other TLR signaling may be associated with T and mDC functions that are suppressed by tumor-infiltrating γδ T cells [89]. Naturally occurring Treg cells require the TICAM-1 pathway in Treg and mDCs for migration to inflamed nests (Fig. 2b), where the MyD88 pathway would restrain their suppressive functions [90]. CD8(+) DEC-205/CD205(+) DCs, but not the CD8(−) DCs, induce functional Foxp3(+) Treg from Foxp3(−) precursors in the presence of low doses of Ag [91]. Subsequent inflammatory Th1-type immunity is modulated by induced Treg cells, which also require the TICAM-1 pathway in mDCs [92]. Treg cells infiltrate the tumor mass and exert immunosuppressive effects that promote tumor progression.

Regulation of TICAM-1 as well as the MyD88 pathway in mDCs may down-regulate Treg in cancer patients [93]. Treg induction is sustained by mDCs with lower maturation stage [94] and what region of TICAM-1 participates in Treg induction remains unknown.

Extrinsic versus intrinsic inflammation for danger signal

PAMPs usually trigger initial or early inflammation around tumors and immune cells in an extrinsic fashion. When tumor cells are damaged through extrinsic inflammation, the destructed cells release cytosolic and nuclear constituents. Inflammation is also promoted by these intrinsic nuclear products including HMGB1, uric acids, S100 proteins, cathelicidins, ATP/adenosine and other nucleosomal proteins [95–98]. These molecules are derivatives of nucleic acids or often have DNA/RNA-binding domains. RNA, DNA and other nucleic acids of host origin also act as danger signals [1, 99]. They are released from damaged host cells or tumors and cause long-lasting inflammation [1, 99]. Recently, they have been named danger-associated molecular pattern (DAMP) or alarmin. Since tumor cells frequently undergo cell death by either apoptosis or necrosis, many cytosolic or nuclear factors are liberated from tumor nests. Tumor progression and reciprocal inflammation involve complicated episodes. We could promote tumor damage followed by DAMP liberation by radiation and/or chemotherapy [100, 101]. Recent reviews infer that electrochemotherapy (ECT) and CpG ODN administration to cancer patients synergistically induce a significant increase of the local effect and a systemic T-dependent antitumor response [100], and that some chemotherapeutic agents with immunostimulatory capacity may facilitate establishing combined chemo-immunotherapy strategies [101]. We should like to clarify these tumor-associated events and responses at a molecular level in order to develop appropriate strategies for the regulation for immune systems in cancer patients. Elucidation of the nucleic acid-recognition systems is essentially required for this purpose. Fundamental issues presented here would hopefully be useful for the development of cancer immunotherapy.

Perspectives

Cancer is a condition in which many immune-related cells form a network in concert with tumor cells. Immune aberrance is an alternative result of tumor growth. APCs and tumor cells exhibit a tight response to innate immune stimulation to alter the balance of tumor tolerance [102].

Cancer stem cells are believed to generate sibling cancer cells. These stem cells are usually vulnerable to irradiation, and their maintenance relies heavily on the gene repair system. It is not known what kinds of RNA sensors and their signaling pathways these stem cells are equipped with [103]. The events that occur in tumor and immune systems upon stem cell modulation by RNA require further study.

Vascular endothelial cells in solid tumors are cytogenetically abnormal. Unlike normal endothelial cells which remain diploid in long-term culture, the aneuploidy of tumor endothelial cells is exacerbated in culture. Tumor-associated endothelial cells upregulate many genes including the epidermal growth factor receptor (EGFR) gene. Accordingly, these cells are highly sensitive to EGF. Endothelial cells usually have a stock of surface-expressed TLR3, which can sense a small RNA duplex structure [104]. Targeting of tumor endothelial cells by immune effector cells may be a possible therapeutic strategy in anti-angiogenic therapy.

In immunological terms, our trials were aimed at elucidating the mechanisms by which mDCs select the mode of activation for various effectors. Results from studies on the dsRNA recognition system, indicated that the properties of PAMP and repertoires of host receptors critically affect these processes. Another issue is how most effective effector cells are induced in a case-dependent manner for tumor remission in patients. This review provides guidelines for the development of specific effector cells by selecting dsRNA receptors. Current knowledge on the TICAM-1 pathway could be directly applied to cancer immunotherapy.

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Original Articles

Innate Immune Therapy with a Bacillus Calmette-Guérin Cell Wall Skeleton After Radical Surgery for Non-Small Cell Lung Cancer: A Case–Control Study

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Abstract

Purpose. We investigated whether adjuvant immunotherapy with Bacillus Calmette-Guérin (BCG) cell wall skeleton (CWS) and surgical resection was better than resection, with or without other adjuvant therapy, for patients with non-small cell lung cancer (NSCLC).

Methods. The case group comprised 71 patients who underwent radical surgery for NSCLC, followed by BCG-CWS immunotherapy, with follow-up data available. The case–control study was designed with one control selected for each case-group patient. Each control was matched by pathological stage and year of birth (±5 years). BCG-CWS 200 μg was inoculated intracutaneously in the upper arm four times per week (sensitization phase); then at 4-week intervals (therapeutic phase).

Results. The case-group patients received 45 ± 22.6 (average ± SD) cycles of BCG-CWS inoculation. Overall 5-year and 10-year survival rates were 71% and 61% for the case-group patients, and 63% and 43% for the control-group patients. The survival rate of the case group was better than that of the control group (not significant; $P = 0.114$). The same trend was seen in the patients with stage III or N+ NSCLC (not significant; $P = 0.114$, $P = 0.168$). There were no life-threatening adverse events.

Conclusions. BCG-CWS immunotherapy seemed to improve survival after resection of NSCLC, especially locally advanced NSCLC. Moreover, this immunotherapy did not compromise quality of life during treatment.

Key words Bacillus Calmette-Guérin cell wall skeleton · Immunotherapy · Non-small cell lung cancer · Surgical resection · Case–control study

Introduction

The human immune system consists of innate and acquired arms. Recent advances in the field of tumor immunology have revealed two novel findings in these two systems: first, most solid tumors express tumor-associated antigens (TAAs) which are rooted in the aberrance of tumor-related genes;[1] and second, activation of the innate immune system before the acquired system is indispensable for full activation of lymphocyte effectors, or cell-mediated immunity.[2] Considering the former issue, immunotherapy for cancer has been designed with TAA peptides and many cytokines, and this augments lymphocyte-based therapies.[3] Rosenberg et al. challenged clinical trials of a peptide vaccine therapy in which a variety of TAAs were administered to melanoma patients. However, the overall rate of remission (including incomplete remission) was only 2.6%.[4] They used peptide vaccines without adjuvant conjugated, or only with aluminum (non-Toll-like receptor (TLR)-directed adjuvant). These results suggest that innate immunity must be stimulated before the induction of acquired effectors to raise antitumor therapeutic potential.

Microbial components that activate the host innate immune system have been designated as adjuvants. Adjuvants are often used for immunization with pure antigens (Ag) for effective induction of antibody (Ab) production, cytotoxic T cells (CTL), and natural killer (NK) cell activation.[5] Many adjuvants have been identified as ligands for microbial pattern-recognition recep-

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