Review Article

Iran J Ped Hematol Oncol. 2017, Vol 7.No 3, 180-192

The Efficacy of Toll-Like Receptors in Awakening Dendritic Cell/Natural Killer Cell System for Eradication of Tumors

Fatemeh Pourrajab PhD [1], Parisa Yazdani Elahabadi MD[2], Seyedhossein Hekmatimoghaddam MD [3, 4,*]

1. Department of Biochemistry and Molecular Biology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
3. Hematology and Oncology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
4. Department of Laboratory Sciences, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

*Corresponding author: Department of Laboratory Sciences, School of Paramedicine, Emam Reza Complex, Daneshjoo Blvd, Yazd, Iran. E-mail: shhekmati2002@yahoo.com

Received: 24 January 2016 **Accepted:** 28 September 2016

Abstract

Natural killer (NK) cells are effector cells of the innate immune system that exert direct cytotoxic functions. Ubiquitously-expressed toll-like receptors (TLRs) have been recognized as one of the major components promoting dendritic cell (DC) maturation, which may induce polarized immune responses beneficial to cancer immunotherapy. TLR-activated NK cells and DCs are prerequisite for robust activation of the innate immune system against tumors. Recently, some medical research and clinical trials have proposed NK cells as a new therapy and potential strategy in both children and adults with those cancers which cannot be cured with the usual treatment modalities. As an example, the importance of DC/NK antitumor immunity in the outcome of breast and other cancers is recently recognized. Therefore, considering strategies which exploit TLR-mediated immunity in concordance with DC/NK system holds strong potential for cancer therapy. This review addresses the current knowledge about the potential role of TLR in tumor immunotherapy.

Key words: Dendritic Cell, Immune System, Natural Killer Cell, Toll-like Receptor, Tumor

Introduction

Natural killer (NK) cells are effector cells of the innate immune system that exert direct cytotoxic functions. Ubiquitously-expressed toll-like receptors (TLRs) have been recognized as one of the major components promoting dendritic cell (DC) maturation, which may induce polarized immune responses beneficial to cancer immunotherapy. TLR-activated NK cells and DCs are prerequisite for robust activation of the innate immune system against tumors. Integration of three factors is required for breaking tumor-induced immune tolerance toward optimal antitumor responses; 1) persistent local TLR signaling, 2) activation of interferons (IFNs) and chemokines, and 3) CD8 T cell response. In this scheme, DC/NK cells in the tumor microenvironment are activated to be capable of initiating a systemic antitumor response. This systemic response would be an orchestration of immune cascades involving the sequential effective activation of NK cells, DCs, and CD8+ T cells in the body. Nowadays, developing an immunotherapy by using NK cells in the pediatric patients who cannot be cured with the usual treatment schemes is considered as an “army of rescuers”. Likewise, the importance of DC/NK antitumor immunity in the outcome of breast and other cancers is recently recognized. Thus, considering strategies which exploit TLR-mediated immunity in concordance with DC/NK system holds strong potential for cancer therapy. This review addresses the current knowledge about the potential role of TLR in tumor immunotherapy.

Local TLRs convert tolerogenic DCs into immunogenic DC/NK system

Tumors have evolved a wide repertoire of immune-suppressive mechanisms to interfere with proper DC maturation and further to block the antitumor T-cell response at all possible levels (1, 2). Some researchers have proposed that it is possible to activate the immune-suppressive mechanisms by directly altering the immunity context in which tumor-Ag cross presentation occurs at the effector site, i.e., in the tumor environment and/or in the tumor-draining lymph nodes (2). Data now make it clear that decoding of a presented Ag (damage-associated molecular pattern, DAMP) by the system of TLRs potentially leads to therapeutic responses (3). Accordingly, tolerance to DCs occurs in the absence of TLR ligation. TLRs expressed by DCs control immune functions such as activation, maturation, and migration of cells (4). TLR agonists provide an immunogenic context to break tolerance or wake up weak priming. Indeed, several reports now support the idea that frequent persistent-interval TLR stimulation breaks tolerance against a given Ag, and that these signals can be provided by viruses or synthetic ligands (5).

TLRs are expressed in most mammalian cells, especially DCs, NK cells, macrophages and T cells (6). TLR1, TLR2 and TLR6 mediate cell activation after interaction with other microbial products such as peptidoglycan and DAMPs. For example, TLR3 reacts with double-stranded RNA (dsRNA), TLR4 with lipopolysaccharide and also with DAMPs, TLR5 with flagellin, TLR7 and TLR8 with single-stranded RNA (ssRNA) and imidazoquinolines (7), and TLR9 with unmethylated CpG DNA. TLR7 and TLR9 are selectively expressed by plasmacytoid DCs (pDCs) (8).

DCs are found to reside in human tumors, and are therefore potentially ideal targets for activation with TLR3, TLR7 and TLR9 agonists (9). Interestingly, natural antitumor immunity is often weak, potentially due to the lack of TLR-mediated activation signals within the tumor microenvironment. TLR-activated NK cells and pDCs initiate a robust activation of the innate immune system, which is mediated by a potent adaptive immune response prerequisite for strong adaptive anti-tumor immunity (10, 11). DCs are specialized antigen-presenting cells (APCs) that recognize, acquire, process, and present antigens to resting, naive T cells for induction of antigen-specific immune responses (12). Upon inflammatory stimuli, immature DCs undergo the maturation process by up-regulation of major histocompatibility complex (MHC) II, co-stimulatory molecules such as CD40 and CD80, and cytokine secretion such as IL-12 and IFN-α. Whereby, mature DCs have a potent ability to induce Th1 CD4+ cell responses and stimulate antigen-specific CD8+ cytotoxic T lymphocyte (CTL) responses (13).

Some novel studies have clearly shown that local persistent/interval TLR stimulation breaks immunological tolerance in the immunosuppressive tumor microenvironment. The intra-tumor administration of TLR ligands is therapeutically beneficial: local stimulation of tumor-sensitized T cells could drive antitumor immunity (6). The classical example for this assumption has been referred to Coley's toxin, a *Serratia marcescens* and *Streptococcus pyogenes* bacteria mixture, which is strikingly efficacious when administered intralesionally in a post-surgery setting (14, 15). The efficacy of Coley's toxin is now attributed to the bacterial CpG DNA recognized by TLR9 (14).

Accordingly, effective antitumor NK and CD8 T cell response is possible by frequent persistent/interval administration of TLR ligand directly targeting the receptors within tumors. Persistent delivery of the TLR-ligand mimetic into

solid tumor establishes an immune response for complete tumor resolution or a significant repression, in which CD8 T response and IFN cytokines both play an important role (13). Administering TLR ligands directly at the effector site causes reactivation of tolerated tumor–resistant DCs and T cells in the draining lymph nodes (16). Surprisingly, it is said that tumor resolution is not dependent on systemic dissemination of or tumor infiltration by effector CD8 T cells, but is critically dependent on the reactivation of tumor resident CD8 T responses (9).

Intra-tumoral administration of increasing or decreasing doses of TLR ligands in the animal model has been reported to have no considerable impact on its efficacy. However, in contrast, increasing the frequency of delivery by an interval manner significantly improves the anti-tumor efficacy and results in complete cure of established tumor which will be able to resist re-challenge with the original tumor (6). Data reveal that local activated pDCs are capable of initiating effective and systemic antitumor immunity through the orchestration of an immune cascade involving the sequential activation of NK cells, cDCs (conventional dendritic cell), and CD8+ T cells. Upon TLR9, TLR7 and TLR3 stimulation at the tumor site, pDCs produce large amounts of chemokines CCL3, CCL4, and CCL5 (17, 18) for recruitment of NK cells to the injected sites. Activated NK cells also secrete CCL3, CCL4, and CCL5, which constitute a positive loop to recruit more NK cells and DCs (19). Interestingly, chemokines CCL3, CCL4, and CCL5 are also known to induce migration of activated memory T cells as well as monocytes. Then, the tumor would contain significant population of tumor-infiltrating lymphocytes (20, 21).

Comparatively, tumor DCs are activated through innate receptors TLR3, TLR7 and TLR9 to produce very high amounts of type I IFNs and IL-12 that, in turn, activate monocytes, NK cells, T cells, and cDCs, the latter cross-priming CD8 T cells (22). Even more, TLR9, TLR7 and TLR3-activated cDCs induce strong, spontaneous CTL cross-priming, which in turn leads to regression of both treated tumors and untreated tumors at distant contralateral sites (**Figure 1**) (23). The main stimuli for immature DCs to undergo maturation are TLRs, and these are also the major receptors which render DCs to express co-stimulatory molecules and secrete inflammatory cytokines (24). The induction of DC maturation by TLRs represents an important functional link between innate and adaptive immune responses (12, 25), rendering DCs capable of efficiently interacting with NK cells. NK cells are strongly activated and became capable of producing IFN-γ and tumor necrosis factor-α (TNF-α) and also acquired cytolytic activity in the presence of TLR3, TLR7 and TLR9 ligands which play a crucial role in inducing NK cells to select the best-fit DCs and to facilitate their maturation. Evidence implies the existence of a remarkable cross-talk between NK cells and DCs that may serve as a control switch between innate and adaptive immune responses (26). Accordingly, NK cells can acquire antitumor cytolytic activity even in the absence of effective DC stimulation. However, a limiting factor in this scenario would be the persistent availability of oligonucleotide ligands, necessary to induce cytotoxicity by NK cells (6).

NK cell stimulation renders a positive loop for cytolytic activity against tumor cells due to the induction of their functional activity for releasing IFN-γ and TNF-α (8). IFN-γ produced by NK cells induces up-regulation of TLR expression by monocyte-derived immature DCs. As a consequence, there will be an increase in the number of DCs equipped with high surface density of receptors involved in Ag uptake (27). Accordingly, a DC-derived soluble factor available for TLR-responsive NK cells would be IFN-γ which will determine a different effect on

subsequent functional responses by NK cells (28).

Importantly, stimuli acting on TLR not only activate immature DCs to release IL-12, but also render NK cells to receive triggering signals from tumor-associated molecules (23). NK activation by oligonucleotides is entirely TLR-dependent, which (in the presence of TLR-mediated IL-12) is characterized by (i) de novo expression of activation markers such as CD69 and CD25, (ii) release of various cytokines including IFN-γ and TNF-α, and (iii) up-regulation of antitumor cytotoxicity (29).

The CTL response in the forefront of DC-NK cell system

According to past studies, direct activation of the innate immune system within a tumor is sufficient to arrest the growth of established tumors and even more to promote tumor resolution. The integration of three factors; 1) frequent TLR-ligand delivery and persistent local TLR signaling, 2) activation of IFNs, 3) CD8 T cell response, is required for tumor resolution. The responsiveness of tumors to local treatment is strongly correlated with increased levels of IFN effectors (potent enhancers of MHC class I levels in normal and cancerous tissues), increasing their sensitivity to CTL-mediated killing (8).

IFNs significantly up-regulate the expression of MHC class I in entire tumor. At low level of MHC class I, TLR ligand activates DCs, which promote NK cells recruitment and anti-tumor activation which is followed by an increasing level of MHC class I and hence, CD8 T cell responses at the effector site, when the expression of MHC class I is significantly up-regulated across the tumor (30). Tumor-infiltrating NK cells are capable of potent cytolytic activity against tumor cells that express low levels of MHC class I at the cell surface (31). However, persistent TLR stimulation in the tumor microenvironment breaks tumor-induced CD8 T cell tolerance and optimal antitumor responses which can completely resolute tumor (1). During TLR ligation, the tumor regression is at first due to a direct innate immune effect and reactivation of local pre-existing CD8 T cells, but later the resolution is supported by cross-primed activated CD8 T cells, as well. Importantly, persistence of IFN effectors in the tumor environment enhances cross-priming of naive CD8 T cells involved in eradication of tumors (6).

In summary, TLR- and also pDCs-activated NK cells initiate the generation of T cell-mediated antitumor immunity through several potential mechanisms: 1) NK cells produce a high amount of IFN-γ, and cell–induced tumor lysis causes release of tumor antigens that subsequently are cross-presented by cDCs, in order to prime antigen-specific CTLs, 2) NK cells induce cDC activation to produce pro-inflammatory cytokines and to elicit tumor-specific T cell responses (32, 3). IFN-γ up-regulates MHC class I and class II expression in tumor cells and cDCs, respectively, to enhance both direct presentation and cross-presentation of tumor antigens (33, 4). IFN-γ produced by NK cells also directly stimulates T cell responses (34). Through direct cell-to-cell contact and type I IFNs, DCs promote generation of CD8 T cell responses (35) (See **Figure 1**). In vivo DC-NK cell interactions involve signals from both soluble cytokines and direct cell-to-cell contact (36). Two to three days following the recruitment of NK cells into the tumor microenvironment, cross-priming of tumor antigen-specific CD8 T cells could be detected in tumor microenvironment. Notably, cross-priming of CD8 T cells is dependent upon NK cells, as well as production of perforin and IFN-γ. At the tumor site, a combination of signals from DCs and from recruited NK cells is needed to cross-prime the robust antigen-specific CTL response (23). In this immunogenicity context, TLR-activated pDCs direct the anti-tumor CD8 T cell

responses not only against tumor-associated antigens at the effector site, but also against the metastatic cells in tissues (22).

The interaction between DCs and NK cells can bypass the needs for T helper for priming of antitumor CD8 CTLs. Emerging evidences address the DC-NK cell cross talk in the induction of tumor CTL responses which obviate the need for CD4 T-cell help (9). Generally, two activation pathways exist which induce CTL priming: The classic pathway (37) involves presentation of peptides by DCs to CD4 T cells, and the novel mechanism introduced here, mediated by a positive feedback loop between NK cells and stimulated DCs to produce IL-12, leading to induction of CD8 lymphocytes. IFN-γ and IL-12 are instrumental in the second novel T helper cell-independent pathway that links the DC-NK cell cross talk to CTL immunity (38-40).

Different mechanisms by TLR ligands for awakening DC-NK system

Based on DC-NK cell system potential, a rational immunization design should be used (involving several different components of the immune system) in order to maximize the priming of CTL responses (41).

The remarkable ability of TLR9/CpG in induction of strong and rapid CD8 CTL responses in cancerous patients through marked CpG-motifs may serve as a promising therapy (42). Accordingly, TLR9/CpG signaling can convert immature, tolerogenic DCs into mature, immunogenic, IL-12/IFN-γ- and IL-6/IL-12/IL-15/IFN-γ-secreting ones, capable of stimulating NK cell and CD8 CTL responses, for antitumor immunity. TLR9/CpG signaling mainly stimulates enhanced IFN-γ/NK /CD8 T cell responses, further leading to induction of efficient antitumor immunity by CD4 Th1/Th17 responses. Complete repression of tumor and metastasis is found to be associated with immunity-inducing CD4 Th17 cells (43). In association with IFN-γ, immunity-inducing CD4 Th17 cells are shown to eradicate established prostate tumors and to inhibit growth of well-established melanoma (24).

Although CD4 Th17 regulatory cells secreting IL-17 and IL-21 have been linked to antitumor immunity, but prolonged TLR9/CpG signaling could lead Th17 regulatory cells to diminish cytokine secretion, stimulatory effect, and thereby antitumor immunity effects. Prolonged (48 h) *in vitro* CpG treatment dramatically diminishes cytokine secretion, stimulatory effect, and thereby antitumor immunity effects (44). The reason may be DCs which induce different types of immune responses at different developmental stages (24).

In summary, TLR9/ pDC-NK cell system starts a potential antitumor immunity by mediating NK /CD8 T cell-cytokine activation within the tumor microenvironment. Additionally, TLR9/DC-NK cell system induces robust spontaneous CTL cross-priming against multiple solid tumor antigens, leading to regression of both treated tumors and untreated tumors at distant contralateral sites. This T cell cross-priming is mediated by early recruitment and activation of NK cells/cDCs at the tumor site. NK cell recruitment is mediated by CCR5 via chemokines secreted by pDCs, and optimal IFN-γ production by NK cells is also mediated by OX40L expressed by pDCs.

Then, activated pDCs are capable to initiating an effective antitumor response, systemically. This systemic response would be an orchestration of immune cascades that involving the sequential effective activation of NK cells, DCs, and CD8+ T cells in the body (23).

Immunostimulatory RNAs elicit an efficient *in vivo* anti-tumor NK response through TLR7/TLR8 which selectively inhibits growth of MHC class I-negative tumors but not growth of MHC class I-

expressing tumors, with enhanced IFN-γ production and increased cytotoxicity, an activated NK cell phenotype (45). TLR7/TLR8 is mainly expressed by human myeloid DCs and monocytes whose activation is essential for Ag presentation and for initiation of immune responses against tumors (30, 46-48).

A single in vivo injection of immune-stimulatory ssRNAs rapidly stimulates NK cells to produce IFN-γ. Activation is mediated by DC-secreted factors, and cell-cell contact is not necessary. The effector functions following RNA treatment are regulated by IFN-γ production which is dependent on IL-12, whereas cytotoxicity depends on type I IFN (49). IFN-γ is a crucial mediator of antitumor immunity in experimental models, and elevated levels of IFN-γ were associated with favorable disease outcome in several clinical studies (50, 51). Two main mechanisms used by NK-mediated antitumor immunity are IFN-γ production and direct cytotoxicity (29).

As with TLR9/CpG, the effector functions are dependent on stimulated DCs to produce IL-12 and type I IFN, the most important abundant cytokines made by activated DCs (52, 53). In a positive loop, INF-γ is involved in priming endogenous DCs for IL-12 production, to achieve efficient protection against tumors (54).

RNA oligonucleotides possess an important therapeutic potential for the treatment of NK-sensitive tumors by triggering the activation of NK cells and the induction of cytokine production by DCs. In addition to their direct anti-tumor efficacy, NK cells favor the generation of CTL through IFN-γ production (41, 55). This mechanism could provide additional ability to induce an Ag-specific CTL response (45). Combined activation of CTL and NK cells by RNA-based therapies eliminating both MHC-negative and MHC-positive tumor cells would prevent tumor immune escape (56).

Local TLR3 activation will augment and sustain regression of large solid tumors through type I and II IFN-as well as DC-dependent mechanisms. Tumor resolution is mainly mediated by type I and II IFNs induced by TLR3 ligand (57, 58). Activation of pre-existing tumor-sensitive CD8s by local delivery of TLR3 ligands is sufficient in promoting significant antitumor responses. As CpG/TLR9 treatments, type I and II IFNs also have an important role in promoting cross-priming of CD8 T cells (59, 60), with IFN-αβ directly enhancing CD8 effector expansion, survival and memory transition (61, 62). Responsiveness to local treatment has been directly correlated to type I and II IFNs, as potent enhancers of MHC class I levels in normal and cancerous tissues, increasing their sensitivity to CTL mediated killing (57, 63). These functions have also been ascribed to IFN-inducing TLR9/TLR7 (64, 65).

Additionally, sequential activation of TLR4 and TLR9/TLR3 and their signaling crosstalk amplifies the activation of macrophages and DC-NK system (66). These findings demonstrate that tumor-derived or exogenous DAMP protein HSP70 taken up by human immature DCs are able to augment pro-inflammatory cytokines IL-1β, IL-12, and TNF-α, besides IFN-γ (67).

It is found that TNF-α, the well-known pleiotropic pro-inflammatory cytokine, is acutely generated by innate cells upon TLR2/4 stimulation (68), and activates endothelial cells and promotes leukocyte infiltration leading to local inflammation mainly through TNFR1 (69). In tumors, TNF-α is triggering apoptosis through Fas-associated protein with death domain (FADD)/caspase8-dependent apoptotic pathway, also via cross-talking with TLR2 (69, 70).

There is a synergistic cooperation between signals of TLR2/4 and TNF-α in the immune cells, mainly through the downstream adaptors tumor necrosis factor receptor type 1-associated death domain (TRADD)/tumor necrosis factor receptor-

associated factors (TRAFs)/NF-ĸB and mitogen-activated protein kinases (MAPK) (71). It is noteworthy that a route of inflammatory amplification signals is initiated by TLR2/4 stimulation, which ends at synergy with TNF responses (72) (**Figure 2**).

TLR2/4-HSP70 interaction exerts potent immunoregulatory effects to up-regulate the expression of pro-inflammatory cytokines through the MyD88/interleukin-1 receptor-associated kinase family (IRAK)/NF-ĸB signal transduction pathway (67, 72).

TLR4/2 signal transduction needs MyD88 to recruit members of IRAK, followed by the activation of TRAF6, switching on the MAPK and NF-ĸB signaling pathways which results in production of pro-inflammatory cytokines and type I IFNs (73, 74). Alternatively, TLR4/3 mediates signaling through TIR-domain-containing adapter-inducing interferon-β (TRIF)-dependent pathway. TRIF is upstream of the IRAKs activation, resulting in type I, II and III IFNs production (72). TLR3/4 activate TRIF-mediated NF-ĸB pathway via the crucial positive mediators TRADD/TRAF3 (74).

Additionally, TLR2/4 activation triggers an upstream membrane-associated adaptor protein (MAL) which turns on MyD88-depended PI3K/Akt (phosphatidylinositide 3-kinase/Protein kinase B (PKB)) pathway (75). TLR2/4 can sequentially activate MAL-MyD88 and TRAM-TRIF pathways through the cell and endosomal membranes, respectively (69,70).

Several nice reviews during the last years have addressed different aspects of TLR usage (mainly by agonists, either approved or still under clinical trial) for cancer therapy (76-79). The interested reader would benefit from considering them, too.

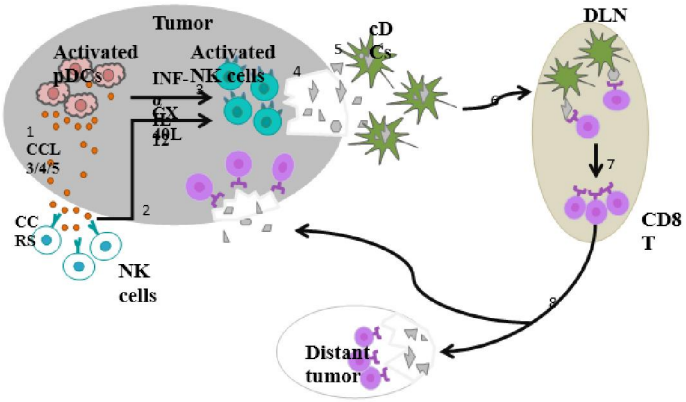

Figure 1. Intra-tumoral ligation of TLRs has remarkable effects in breaking immunogenic resistance and mobilizing a local tolerated/ineffectively-activated T cell repertoire, through the effector function of DC-NK system. In the tumor microenvironment, TLR ligation activates plasmacytoid dendritic cells (pDCs) and NK cells at the effector site. Upon stimulation with TLR9, TLR7 and TLR3, activated cells produce large amounts of type I IFNs/IL-12 cytokines and chemokines CCL3, CCL4, and CCL5, that, in turn, causes recruitment and activation of NK cells, T cells, and conventional DCs (cDCs) in a positive feedback loop. Recruited NK cells are activated to produce IFN-γ and chemokines, in a positive feedback loop through cytokines and cell-cell interactions. Activated NK cells initiate tumor cell killing via enhanced cytolytic activity. Tumor-associated antigens released by NK-mediated tumor destruction are taken up by activated cDCs, which then migrate to tumor draining lymph node (DLN). Cross-presentation of tumor antigens by activated cDCs in DLN leads to effective cross-priming and expansion of tumor antigen-specific T cells. Infiltration of both treated and untreated tumors by cross-primed CD8+ T cells mediates further tumor cell killing and systemic antitumor immunity. [By courtesy of Chengwen Liu et al., Plasmacytoid dendritic cells induce NK cell-dependent, tumor antigen-specific T cell cross-priming and tumor regression in mice. The Journal of Clinical Investigation 2008; 118(3), with some modifications]

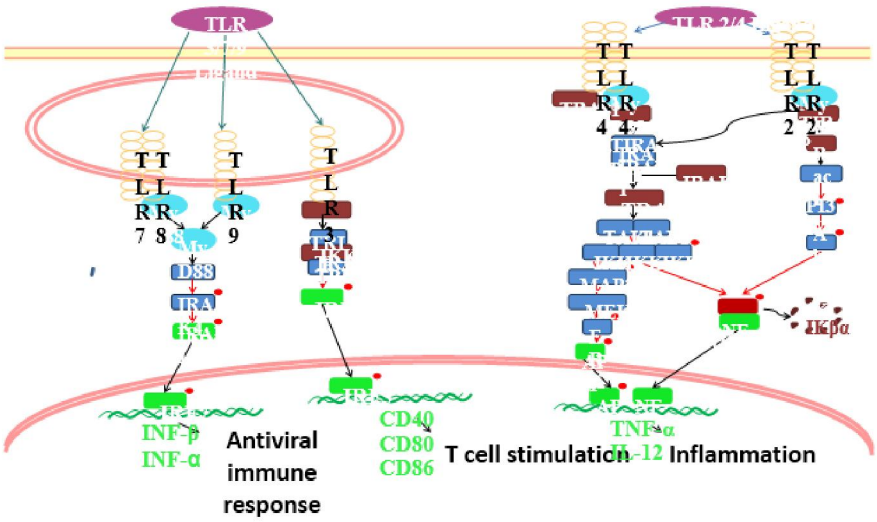

Figure2. Different mechanisms are illustrated by TLR ligands for awakening DC-NK system which result in the eradication of invading tumors. Triggering of various TLRs, or sets of TLRs, activates different intracellular signaling pathways resulting in different repertoires of cytokine genes to be activated. This means that the innate immune system allows cells to react with an immediate and balanced response to invading cells. TLR family plays an instructive role in activation of innate immune as well as the subsequent induction of adaptive immune responses. They recognize specific molecular patterns and triggering inflammatory responses and dendritic cell maturation, which can be used in the eradication of invading tumors. Individual TLRs interact with different combinations of adapter proteins and activate various transcription factors such as NF-KB, activating protein-1(AP-1) and interferon regulatory factors (IFRs), driving a specific immune response. This view outlines the recent advances in the understanding of TLR-signaling pathways and their roles in immune responses against invading tumors (in vivo/in vitro).

Conclusion

Hereby we tried to demonstrate that the inflammatory amplification routes which have been identified in TLR signaling, illustrate the putative mechanism by TLR activation, in which the recruitment of TRAFs into TNF/TRIF-associated signalsome plays a critical role in amplifying inflammatory responses. The cross-talk between TLRs and TNFR introduces a fascinating target for designing new therapeutics to induce immune responses in tumors (72).

It is hoped that recognition of the important role of TLR will help in development of new therapeutic modalities for tumors.

Conflict of interest

All authors declare that they have no conflict of interest.

References

1. Gajewski TF, Meng Y, Blank C, Brown I, Kacha A, Kline J, Harlin H. Immune resistance orchestrated by the tumor microenvironment. Immunol Rev, 2006; 131-45

2. Van Mierlo GJ, Boonman ZF, Dumortier HM, den Boer AT, Fransen MF, Nouta J, van der Voort EI, Offringa R, Toes RE, Melief CJ. Activation of dendritic cells that cross-present tumor-derived antigen licenses CD8+ CTL to cause tumor eradication. J Immunol 2004; 173: 6753-9

3. Trinchieri G, Sher A. Cooperation of Toll-like receptor signals in innate immune defence. Nat Rev Immunol 2007; 7: 179-90

4. Degli-Esposti MA, Smyth MJ. Close encounters of different kinds: dendritic cells and NK cells take centre stage. Nat Rev Immunol 2005; 5: 112-24

5. Yang Y, Huang CT, Huang X, Pardoll DM. Persistent Toll-like receptor signals are required for reversal of regulatory T cell-mediated CD8 tolerance. Nat Immunol 2004; 5: 508-15
6. Currie AJ, van der Most RG, Broomfield SA, Prosser AC, Tovey MG, Robinson BW. Targeting the effector site with IFN-alphabeta-inducing TLR ligands reactivates tumor-resident CD8 T cell responses to eradicate established solid tumors. J Immunol 2008; 180: 1535-44
7. Campos MA, Almeida IC, Takeuchi O, Akira S, Valente EP, Procopio DO, Travassos LR, Smith JA, Golenbock DT, Gazzinelli RT. Activation of Toll-like receptor-2 by glycosylphosphatidylinositol anchors from a protozoan parasite. J Immunol 2001; 167: 416-23
8. Sivori S, Falco M, Della Chiesa M, Carlomagno S, Vitale M, Moretta L, Moretta A. CpG and double-stranded RNA trigger human NK cells by Toll-like receptors: induction of cytokine release and cytotoxicity against tumors and dendritic cells. Proc Natl Acad Sci USA 2004; 101: 10116-21
9. Mohty M, Olive D, Gaugler B. DCs and cancer: a new role for an enigmatic cell. Trends Immunol 2004; 25: 397-8; author reply 8-9
10. Fearon DT, Locksley RM. The instructive role of innate immunity in the acquired immune response. Science 1996; 272: 50-3
11. Medzhitov R, Janeway CA, Jr. Innate immunity: the virtues of a nonclonal system of recognition. Cell 1997; 91: 295-8
12. Banchereau J, Palucka AK. Dendritic cells as therapeutic vaccines against cancer. Nat Rev Immunol 2005; 5: 296-306
13. Langenkamp A, Messi M, Lanzavecchia A, Sallusto F. Kinetics of dendritic cell activation: impact on priming of TH1, TH2 and nonpolarized T cells. Nat Immunol 2000; 1: 311-6
14. Killeen SD, Wang JH, Andrews EJ, Redmond HP. Exploitation of the Toll-like receptor system in cancer: a doubled-edged sword? Br J Cancer 2006; 95: 247-52
15. Tsung K, Norton JA. Lessons from Coley's toxin. Surg Oncol 2006; 15: 25-8
16. Marzo AL, Lake RA, Robinson BW, Scott B. T-cell receptor transgenic analysis of tumor-specific CD8 and CD4 responses in the eradication of solid tumors. Cancer Res 1999; 59: 1071-9
17. Megjugorac NJ, Young HA, Amrute SB, Olshalsky SL, Fitzgerald-Bocarsly P. Virally stimulated plasmacytoid dendritic cells produce chemokines and induce migration of T and NK cells. J Leukoc Biol 2004; 75: 504-14
18. Piqueras B, Connolly J, Freitas H, Palucka AK, Banchereau J. Upon viral exposure, myeloid and plasmacytoid dendritic cells produce 3 waves of distinct chemokines to recruit immune effectors. Blood 2006; 107: 2613-8
19. Walzer T, Dalod M, Vivier E, Zitvogel L. Natural killer cell-dendritic cell crosstalk in the initiation of immune responses. Expert Opin Biol Ther 2005; 1: S49-59
20. Cook DN, Beck MA, Coffman TM, Kirby SL, Sheridan JF, Pragnell IB, Smithies O. Requirement of MIP-1 alpha for an inflammatory response to viral infection. Science 1995; 269: 1583-5
21. Sallusto F, Lenig D, Mackay CR, Lanzavecchia A. Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. J Exp Med 1998; 187: 875-83
22. McKenna K, Beignon AS, Bhardwaj N. Plasmacytoid dendritic cells: linking innate and adaptive immunity. J Virol 2005; 79: 17-27
23. Liu C, Lou Y, Lizee G, Qin H, Liu S, Rabinovich B, Kim GJ, Wang YH, Ye Y, Sikora AG, Overwijk WW, Liu YJ, Wang G, Hwu P. Plasmacytoid dendritic cells induce NK cell-dependent, tumor antigen-specific T cell cross-priming and tumor regression in mice. J Clin Invest 2008; 118: 1165-75

24. Zhang X, Munegowda MA, Yuan J, Wei Y, Xiang J. Optimal TLR9 signal converts tolerogenic CD4-8- DCs into immunogenic ones capable of stimulating antitumor immunity via activating CD4+ Th1/Th17 and NK cell responses. J Leukoc Biol 2010; 88: 393-403
25. Huang Q, Liu D, Majewski P, Schulte LC, Korn JM, Young RA, Lander ES, Hacohen N. The plasticity of dendritic cell responses to pathogens and their components. Science 2001; 294: 870-5
26. Jakob T, Walker PS, Krieg AM, Udey MC, Vogel JC. Activation of cutaneous dendritic cells by CpG-containing oligodeoxynucleotides: a role for dendritic cells in the augmentation of Th1 responses by immunostimulatory DNA. J Immunol 1998; 161: 3042-9
27. Bosisio D, Polentarutti N, Sironi M, Bernasconi S, Miyake K, Webb GR, Martin MU, Mantovani A, Muzio M. Stimulation of toll-like receptor 4 expression in human mononuclear phagocytes by interferon-gamma: a molecular basis for priming and synergism with bacterial lipopolysaccharide. Blood 2002; 99: 3427-31
28. Kerkmann M, Rothenfusser S, Hornung V, Towarowski A, Wagner M, Sarris A, Giese T, Endres S, Hartmann G. Activation with CpG-A and CpG-B oligonucleotides reveals two distinct regulatory pathways of type I IFN synthesis in human plasmacytoid dendritic cells. J Immunol 2003; 170: 4465-74
29. Bourquin C, Schmidt L, Lanz AL, Storch B, Wurzenberger C, Anz D, Sandholzer N, Mocikat R, Berger M, Poeck H, Hartmann G, Hornung V, Endres S. Immunostimulatory RNA oligonucleotides induce an effective antitumoral NK cell response through the TLR7. J Immunol 2009; 183: 6078-86
30. Hornung V, Rothenfusser S, Britsch S, Krug A, Jahrsdorfer B, Giese T, Endres S, Hartmann G. Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. J Immunol 2002; 168: 4531-7
31. Della Chiesa M, Vitale M, Carlomagno S, Ferlazzo G, Moretta L, Moretta A. The natural killer cell-mediated killing of autologous dendritic cells is confined to a cell subset expressing CD94/NKG2A, but lacking inhibitory killer Ig-like receptors. Eur J Immunol 2003; 33: 1657-66
32. Dao T, Gomez-Nunez M, Antczak C, Kappel B, Jaggi JS, Korontsvit T, Zakhaleva V, Scheinberg DA. Natural killer cells license dendritic cell cross-presentation of B lymphoma cell--associated antigens. Clin Cancer Res 2005; 11: 8763-72
33. Van den Elsen PJ, Gobin SJ, van Eggermond MC, Peijnenburg A. Regulation of MHC class I and II gene transcription: differences and similarities. Immunogenetics 1998; 48: 208-21
34. Smyth MJ, Kelly JM. Accessory function for NK1.1+ natural killer cells producing interferon-gamma in xenospecific cytotoxic T lymphocyte differentiation. Transplantation 68: 840-3
35. Beignon AS, Skoberne M, Bhardwaj N. 2003. Type I interferons promote cross-priming: more functions for old cytokines. Nat Immunol 1999; 4: 939-41
36. Walzer T, Dalod M, Robbins SH, Zitvogel L, Vivier E. Natural-killer cells and dendritic cells: "l'union fait la force". Blood 2005; 106: 2252-8
37. Schoenberger SP, Toes RE, Van der Voort EI, Offringa R, Melief CJ. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. Nature 1998; 393: 480-3
38. Fernandez NC, Lozier A, Flament C, Ricciardi-Castagnoli P, Bellet D, Suter M, Perricaudet M, Tursz T, Maraskovsky E, Zitvogel L. Dendritic cells directly trigger NK cell functions: cross-talk relevant in innate anti-tumor immune responses in vivo. Nat Med 1999; 5: 405-11
39. Yu Y, Hagihara M, Ando K, Gansuvd B, Matsuzawa H, Tsuchiya T, Ueda Y, Inoue H, Hotta T, Kato S. Enhancement of

human cord blood CD34+ cell-derived NK cell cytotoxicity by dendritic cells. J Immunol 2001; 166: 1590-600
40. Turner JG, Rakhmilevich AL, Burdelya L, Neal Z, Imboden M, Sondel PM, Yu H. Anti-CD40 antibody induces antitumor and antimetastatic effects: the role of NK cells. J Immunol 2001; 166: 89-94
41. Adam C, King S, Allgeier T, Braumuller H, Luking C, Mysliwietz J, Kriegeskorte A, Busch DH, Rocken M, Mocikat R. DC-NK cell cross talk as a novel CD4+ T-cell-independent pathway for antitumor CTL induction. Blood 2005; 106: 338-44
42. Krieg AM. Development of TLR9 agonists for cancer therapy. J Clin Invest 2007; 117: 1184-94
43. Kryczek I, Wei S, Szeliga W, Vatan L, Zou W. Endogenous IL-17 contributes to reduced tumor growth and metastasis. Blood 2009;114: 357-9
44. Spolski R, Leonard WJ. Interleukin-21: basic biology and implications for cancer and autoimmunity. Annu Rev Immunol 2008; 26: 57-79
45. Bourquin C, Schmidt L, Hornung V, Wurzenberger C, Anz D, Sandholzer N, Schreiber S, Voelkl A, Hartmann G, Endres S. Immunostimulatory RNA oligonucleotides trigger an antigen-specific cytotoxic T-cell and IgG2a response. Blood 2007; 109: 2953-60
46. Heil F, Hemmi H, Hochrein H, Ampenberger F, Kirschning C, Akira S, Lipford G, Wagner H, Bauer S. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science 2004; 303: 1526-9
47. Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science 2004; 303: 1529-31
48. Krug A, Towarowski A, Britsch S, Rothenfusser S, Hornung V, Bals R, Giese T, Engelmann H, Endres S, Krieg AM, Hartmann G. Toll-like receptor expression reveals CpG DNA as a unique microbial stimulus for plasmacytoid dendritic cells which synergizes with CD40 ligand to induce high amounts of IL-12. Eur J Immunol 2001; 31: 3026-37
49. Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. Nat Immunol 2008; 9: 503-10
50. Terme M, Ullrich E, Delahaye NF, Chaput N, Zitvogel L. Natural killer cell-directed therapies: moving from unexpected results to successful strategies. Nat Immunol 2008; 9: 486-94
51. Dunn GP, Bruce AT, Sheehan KC, Shankaran V, Uppaluri R, Bui JD, Diamond MS, Koebel CM, Arthur C, White JM, Schreiber RD. A critical function for type I interferons in cancer immunoediting. Nat Immunol 2005; 6: 722-9
52. Sawaki J, Tsutsui H, Hayashi N, Yasuda K, Akira S, Tanizawa T, Nakanishi K. Type 1 cytokine/chemokine production by mouse NK cells following activation of their TLR/MyD88-mediated pathways. Int Immunol 2007; 19: 311-20
53. Girart MV, Fuertes MB, Domaica CI, Rossi LE, Zwirner NW. Engagement of TLR3, TLR7, and NKG2D regulate IFN-gamma secretion but not NKG2D-mediated cytotoxicity by human NK cells stimulated with suboptimal doses of IL-12. J Immunol 2007; 179: 3472-9
54. Martinez J, Huang X, Yang Y. Direct action of type I IFN on NK cells is required for their activation in response to vaccinia viral infection in vivo. J Immunol 2008; 180: 1592-7
55. Zhou H, Luo Y, Kaplan CD, Kruger JA, Lee SH, Xiang R, Reisfeld RA. A DNA-based cancer vaccine enhances lymphocyte cross talk by engaging the NKG2D receptor. Blood 2006; 107: 3251-7
56. Diefenbach A, Jensen ER, Jamieson AM, Raulet DH. Rae1 and H60 ligands of the NKG2D receptor stimulate tumour immunity. Nature 2001; 413: 165-71
57. Oberg K. The action of interferon alpha on human carcinoid tumours. Semin Cancer Biol 1992; 3: 35-41

58. McBride S, Hoebe K, Georgel P, Janssen E. Cell-associated double-stranded RNA enhances antitumor activity through the production of type I IFN. J Immunol 2006; 177: 6122-8
59. Le Bon A, Tough DF. Links between innate and adaptive immunity via type I interferon. Curr Opin Immunol 2002; 14: 432-6
60. Le Bon A, Etchart N, Rossmann C, Ashton M, Hou S, Gewert D, Borrow P, Tough DF. Cross-priming of CD8+ T cells stimulated by virus-induced type I interferon. Nat Immunol 2003; 4: 1009-15
61. Le Bon A, Durand V, Kamphuis E, Thompson C, Bulfone-Paus S, Rossmann C, Kalinke U, Tough DF. Direct stimulation of T cells by type I IFN enhances the CD8+ T cell response during cross-priming. J Immunol 2006; 176: 4682-9
62. Kolumam GA, Thomas S, Thompson LJ, Sprent J, Murali-Krishna K. Type I interferons act directly on CD8 T cells to allow clonal expansion and memory formation in response to viral infection. J Exp Med 2005; 202: 637-50
63. Halloran PF, Urmson J, Van der Meide PH, Autenried P. Regulation of MHC expression in vivo. II. IFN-alpha/beta inducers and recombinant IFN-alpha modulate MHC antigen expression in mouse tissues. J Immunol 1989; 142: 4241-7
64. Durand V, Wong SY, Tough DF, Le Bon A. IFN-alpha/beta-dependent cross-priming induced by specific toll-like receptor agonists. Vaccine 2006; 2: S2-22-3
65. Durand V, Wong SY, Tough DF, Le Bon A. Shaping of adaptive immune responses to soluble proteins by TLR agonists: a role for IFN-alpha/beta. Immunol Cell Biol 2004; 82: 596-602
66. De Nardo D, De Nardo CM, Nguyen T, Hamilton JA, Scholz GM. Signaling crosstalk during sequential TLR4 and TLR9 activation amplifies the inflammatory response of mouse macrophages. J Immunol 2009; 183: 8110-8
67. Asea A, Rehli M, Kabingu E, Boch JA, Bare O, Auron PE, Stevenson MA, Calderwood SK. Novel signal transduction pathway utilized by extracellular HSP70: role of toll-like receptor (TLR) 2 and TLR4. J Biol Chem 2002; 277: 15028-34
68. Marino MW, Dunn A, Grail D, Inglese M, Noguchi Y, Richards E, Jungbluth A, Wada H, Moore M, Williamson B, Basu S, Old LJ. Characterization of tumor necrosis factor-deficient mice. Proc Natl Acad Sci U S A 1997; 94: 8093-8
69. Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. Nat Rev Immunol 2003; 3: 745-56
70. Schneider-Brachert W, Tchikov V, Neumeyer J, Jakob M, Winoto-Morbach S, Held-Feindt J, Heinrich M, Merkel O, Ehrenschwender M, Adam D, Mentlein R, Kabelitz D, Schutze S. Compartmentalization of TNF receptor 1 signaling: internalized TNF receptosomes as death signaling vesicles. Immunity 2004; 21: 415-28
71. Sasai M, Tatematsu M, Oshiumi H, Funami K, Matsumoto M, Hatakeyama S, Seya T. Direct binding of TRAF2 and TRAF6 to TICAM-1/TRIF adaptor participates in activation of the Toll-like receptor 3/4 pathway. Mol Immunol 2010; 47: 1283-91
72. Chang YL, Chen TH, Wu YH, Chen GA, Weng TH, Tseng PH, Hsieh SL, Fu SL, Lin CH, Chen CJ, Chu CL, Chio, II, Mak TW, Chen NJ. A novel TLR2-triggered signalling crosstalk synergistically intensifies TNF-mediated IL-6 induction. J Cell Mol Med 2014; 18: 1344-57
73. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol 2010; 11: 373-84
74. Cao Z, Xiong J, Takeuchi M, Kurama T, Goeddel DV. TRAF6 is a signal transducer for interleukin-1. Nature 1996; 383: 443-6

75. Fitzgerald KA, Palsson-McDermott EM, Bowie AG, Jefferies CA, Mansell AS, Brady G, Brint E, Dunne A, Gray P, Harte MT, McMurray D, Smith DE, Sims JE, Bird TA, O'Neill LA. Mal (MyD88-adapter-like) is required for Toll-like receptor-4 signal transduction. Nature 2001; 413: 78-83

76. Shi M1, Chen X, Ye K, Yao Y, Li Y. Application potential of toll-like receptors in cancer immunotherapy: Systematic review. Medicine (Baltimore) 2016 Jun; 95(25): e3951

77. Wang JQ, Jeelall YS, Ferguson LL, Horikawa K. Toll-Like Receptors and Cancer: MYD88 Mutation and Inflammation. Front Immunol 2014; 5: 367.

78. Pradere JP, Dapito DH, Schwabe RF. The Yin and Yang of Toll-like receptors in cancer. Oncogene 2014 Jul 3; 33(27): 3485-95

79. Kaczanowska S, Joseph AM, Davila E. TLR agonists: our best frenemy in cancer immunotherapy. J Leukoc Biol 2013 Jun; 93(6): 847–863.