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An adjuvant role of *in situ* dendritic cells (DCs) in linking innate and adaptive immunity

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1. ABSTRACT

Dendritic cells (DCs) work as natural adjuvants to elicit T cell immunity. Though DCs have been widely used in immunotherapy, little is known about their number and function in patients with cancer or autoimmune disease. In recent studies, antigen has been targeted to DCs through DC-specific receptors, such as DEC205, the mannose receptor and dying cell receptors. However, antigen captured by DCs in the absence of danger signals induces tolerance. Therefore, the duration and/or magnitude of danger signals plays a crucial role in generating an immunogeneic response. Various danger signals, i.e., pathogen-associated molecular pattern (PAMP), damage-associated molecular pattern (DAMP) and the activation of innate lymphocytes, serve as maturation signals for DCs. An immunotherapeutic approach which delivers both maturation signals and antigen to DCs would link the innate and adaptive arms of the immune system for a more effective and global immune response. It is therefore crucial to determine optimal conditions for antigen delivery to DCs in an environment suited to maximally stimulate the immune system.

2. INTRODUCTION

Activation and maturation enhances the antigen-presenting capacity of dendritic cells (DCs) by upregulating the expression of co-stimulatory molecules and chemokine receptors that promote migration to nodal T cell areas. Targeting of antigen to DC receptors without providing proper maturation stimuli results in tolerance in mice. This strategy can be used in disease states where silencing the immune system to specific antigens would benefit the patient, i.e. allergy, autoimmunity, transplant rejection and chronic inflammatory diseases. By contrast, vaccination strategies to induce immunity against cancer or infectious diseases need to include a means of maturing the targeted DC in order to elicit an adaptive immune response. Systemic application of adjuvants such as α-galactosylceramide (α-GalCer) (1), CD40-specific antibody (2) and Toll-like receptor (TLR) ligands (3) enhance $CD8^+$ T cell responses to co-administered antigens. When applying maturation stimuli, two factors, i.e., timing and route of administration, are of crucial importance.

TLR on DCs bind microbial constituents, such as protein, RNA and DNA, expressed on infected cells. Antigen plus TLR ligand serves as a danger signal,

triggering DC maturation and ultimately leading to the induction of T cell immunity. Recent studies show that innate lymphocytes, such as natural killer cells (NK cells), invariant natural killer T cells (iNKT cells), and γδ T cells, activate and mature DCs after detecting infected or transformed cells (4).

In the wake of encouraging preliminary studies, innate lymphocyte activation as well as TLR stimuli can be further evaluated as adjuvants for DC-based vaccines.

3. ADJUVANT EFFECTS OF INNATE IMMUNITY

3.1. Adjuvant effects of toll-like receptor (TLR) ligands

DC maturation acts as a sensor, linking different innate responses with different adaptive ones. We will characterize DC maturation induced by TLRs in terms of adjuvant effects (Figure 1). Due to their role in the development of the Th1-immune response, TLR agonists have been heavily explored as immunotherapeutics and vaccine adjuvants. One group of TLRs, consisting of TLR1, 2, 4, 5, 6 and 11, is located on the cell surface and is responsible for the detection of external pathogen structures, like lipopeptides (TLR1, 2 and 6), LPS (TLR 2 and 4), flagellin (TLR5) and profilin (TLR11) (3, 5). The other group, formed by TLR 3, 7, 8 and 9, is located in the endoplasmic reticulum and endosomal-lysosomal compartment. There, they recognize bacterial or viral nucleic acid (3).

3.1.1 Adjuvant effects of LPS

TLR4, upon recognition of its ligand LPS, induces the production of inflammatory cytokines, including IL-6, TNF-α, and IL-12 through the adaptors MyD88 and TIRAP (3). TLR4 signaling can also induce activation of IRF-3 and production of IFN-α/β through the adaptors TRAM and TRIF (3). MyD88 is absolutely required for the production of inflammatory cytokines by DCs, but not for their maturation, as is evidenced by the normal phenotypic maturation of DCs in $MyD88^{-/-}$ mice (6). However, Pasare *et al* showed TLR-induced DC maturation and migration to the lymph nodes is not sufficient for T cell activation in $MyD88^{-/-}$ mice (7). Recently, it was shown that LPS induces IL-10-producing Tr1 cells which suppress the activation of $CD8^+$ T cells (8).

3.1.2. Adjuvant effects of CpG

CpG-DNA has proven to be one of the strongest Th1 inducing adjuvants known *in vivo*. In animal models, stimulation of TLR9 with CpG has been shown to increase the immunogenecity of vaccines using peptides, DNA, tumor cells (9). The immunostimulatory sequence (ISS) of CpG in combination with antigen (Ag) conjugates (CpG-Ag complex) has been reported to have robust immune effects in a number of different model systems. Antigen-immunostimulatory oligonucleotide conjugates may be more effective in generating adaptive immunity than co-injection of antigen plus ISS (9). Heit *et al* demonstrated that OVA covalently linked to CpG-DNA (CpG-OVA complex) is efficiently internalized by DCs via DNA receptor-mediated endocytosis. And then, it is translocated to lysosomal-associated membrane protein 1 (LAMP-1)-positive endosomal-lysosomal compartments, which have recently been shown to participate in cross presentation (10). Probably, both the antigen and TLR ligand need to co-localize within the same phagosome for efficient MHC class II antigen presentation to occur. This CpG-OVA complex-mediated reaction has the ability to link innate and adaptive immunity.

3.1.3. Adjuvant effects of RNA

Double stranded RNA (dsRNA) acts as a viral-associated recognition motif for TLR3. TLR7 in mice and TLR7 and TLR8 in humans have recently been shown to recognize guanosine- or uridine-rich single-stranded or synthetic RNA (ssRNA) from viruses such as human immunodeficiency virus, vesicular stomatitis virus, and influenza virus (11). Schulz *et al* showed that immunization of mice with virus-infected cells or cells containing synthetic dsRNA triggered TLR3 signaling of $CD8^+$ DCs, leading to a striking increase in CTL cross-priming (12).

3.1.4. Enhancement of the adjuvant effects by combination of TLR-ligand stimuli

Approaches have been developed for harnessing the effects of TLR ligands. One is combining different types of TLR ligands for use as DC maturation stimuli. For example, TLR3 and TLR4 act in synergy with TLR7, TLR8, and TLR9 to induce IL-12 and IL-23 production by DCs (13). Triggering TLR and CD40 leads to potent cellular immunity dependent on IFN-α/β production and the expression of CD70 on DCs (14, 15). Furthermore, TLR binding to protein antigens, such as R848 (which signals through TLR7 and TLR8) (16), CpG (which signals through TLR9) (10), and profilin (which signals through TLR11) (5), has been reported to enhance presentation of antigen to T cells.

3.1.5. Differences of TLR expression on human and murine DC subsets

Murine plasmacytoid and myeloid DCs express both TLR3 (double-stranded RNA receptor) and TLR9 (ISS DNA receptor). Binding of either receptor to its appropriate ligand results in DC maturation (17). In contrast, TLR9 and TLR7 are preferentially expressed on human plasmacytoid DCs (pDCs), whereas TLR8 is expressed on myeloid DCs and monocytes in humans and macaques (17). Wille-Reece *et al* demonstrated in rhesus macaques that HIV gag protein conjugated to a TLR7/8 agonist improves the magnitude and quality of Th1 and $CD8^+$T cell responses to HIV gag protein more than co-injection of unconjugated gag protein and TLR7/8 agonist (16, 18). They also showed a TLR7/8 agonist elicited greater adjuvant effects than a TLR9 agonist. In contrast, a separate group found rhesus macaque monkeys primed with an immunodeficiency virus DNA vaccine had a more vigorous response if they were given the TLR9 ligand (CpG DNA type B) instead of the TLR7/8 ligand (3M compound 003) (19). The immunized animals demonstrated increased numbers of antigen-specific $CD8^+IFN\text{-}\gamma^+$ T cells as well as polyfunctional $CD8^+$ T cells producing IFN-γ, TNF-α and IL-2. These results indicate an indirect mechanism of cross-priming secondary to the release of pro-inflammatory cytokines by pDC.

3.2. Adjuvant effects through other pathways such as Nod-like receptor (NLRs) and retinoic acid-inducible gene I (RIG-1)-like helicases (RLHs)

In addition to TLR, two other families of pattern recognition receptors (PPRs) have recently been identified, Nod-like receptors (NLRs) and retinoic acid-inducible gene I (RIG-1)-like helicases (RLHs).

NLRs are a family of 22 receptors recognizing intracellular microbial components separated into two large protein subclasses: Nod (Nod1-5) and NALPs (NACT, leucin-repeat, and pyrin-domain-containing proteins 1-14) (20). Nod1, Nod2, and NALP3 are activated by muropeptides, small bacterial peptidoglycan fragments derived from Gram-positive and Gram-negative bacterial cell walls (20). NALP3 can also be activated by endogenous danger signals, such as uric acid and ATP, released from dying cells (21). Upon recognition of PAMPs, NLRs undergo a conformational change and trigger the signaling pathways that yield proinflammatory cytokines, such as IL-1β and IL-18. A recent study showed that Nod1 stimulation alone induced antigen-specific immunity with a predominant Th2 polarization profile whereas in conjunction with TLR stimulation, Nod1 played a crucial role for priming Th1 as well as Th17 immune pathways *in vivo* (22). NLR expression on human DCs is largely unknown, except for the expression of Nod1 and Nod2 in the cytosol of macrophages and DCs. Polymorphism or mutations in NLRs are correlated with susceptibility to autoinflammatory diseases (20), such as the association of Nod2 with the development of Crohn's disease and familial sarcoidosis (23).

Three homologous DExD/H box RNA helicases were identified as cytoplasmic sensors of virally derived dsRNA or ssRNA. Retinoic-acid-inducible gene I (RIG-1) and melanoma-differentiation–associated gene 5 (MDA5) share two N-teminal caspase recruitment domains (CARDs) followed by an RNA helicase domain. In contrast, LGP2 lacks a CARD (24). It has recently been shown that MDA5, but not RIG-1, was responsible for the response to poly I:C stimulation. Trumpfheller *et al.* showed that poly I:C as an adjuvant in DC-targeted antigen delivery system using anti-DEC-HIV gag p24 leads to a long-lived and protective Th1 $CD4^+$ T cell response dependent on both TLR3 and MDA5 (25).

3.3. Adjuvant effects of activated innate lymphocytes

DC maturation by innate lymphocytes depends on the activation of NK, iNKT and γδ T cells. These cells can be activated by synthetic ligands as well as by self-molecules, such as MHC class I-related proteins (4), lysosomal glycolipids (26) and small molecular metabolites (eg. aminobiphosphonate), respectively (Figure 1). We will focus on NK cell and iNKT cell activation in this part.

3.3.1. Adjuvant effects of NK cells

NK cells directly recognize their targets via two groups of receptors, activating and inhibitory (4, 26). NK receptors known for detecting infection or transformation are Ly49H, NKG2D (4) and the natural cytotoxicity receptors (NCRs), which are the main activating receptors of NK cells. Both in humans and mice, NKG2D recognizes self-molecules expressed on the cell surface upon transformation or infection. In humans, the self molecules are the MHC class I chain-related proteins A and B (MICA and B) and UL16 binding proteins (ULBP1-3), and in mice, the retinoic acid early inducible 1 protein family (Rae1α–ε), H60 and murine ULBP-like transcript 1 (MULT1). NK cell activation is further enhanced by the absence of NK cell inhibition. The inhibitory NK receptors of the CD94/NKG2, Ly49 and killer cell immunoglobulin-like receptor (KIR) families recognize classical and non-classical MHC class I molecules (26).

Activated NK cells are able to mature DCs both *in vitro* and *in vivo.* NK cell-mediated maturation of DCs results in phenotypic maturation, IL-12 and TNFα secretion, and the induction of protective $CD8^+$ T cell responses (27, 28). NK cells mature DCs by a combination of TNF-α secretion and cell-cell contact (27, 28). In addition, NK cell activation is able to polarize DC-initiated T cell responses towards a Th1 pattern, primarily via IFN-γ (29).

3.3.2. Adjuvant effects of iNKT cells

We demonstrated iNKT cells activated by α-GalCer can serve as a DC maturation stimulus *in vivo* (1). In our study, an intravenous injection of a single standard dose of soluble α-GalCer was given, and subsequent DC maturation was measured by several criteria. We observed a marked increase in costimulatory molecules (CD40, 80, 86, B7-DC) and MHC class II to a level equal that seen after stimulation with TLR ligands, such as LPS or CpG (1). These cells also produced cytokines, such as TNF-α and IL-12, at levels comparable to that of mature DCs (30). Fixed splenic cells from mice given α-GalCer were strong stimulators of T cell proliferation in the mixed leukocyte reaction as opposed to fixed splenic cells from mice not given α-GalCer, demonstrating DC maturation *in vivo*. From this, we established that most DCs in a mouse spleen were in an immature state but could mature in response to α-GalCer. Mice were injected with cell-associated ovalumin (OVA) as antigen with or without α-GalCer. Only mice given OVA in combination with α-GalCer developed Th1 $CD4^+$ and $CD8^+$ T cell responses and demonstrated resistance to challenge with an OVA-expressing tumor (1). This was the first demonstration that activation of innate lymphocytes *in vivo* could lead to DC maturation and subsequent adaptive immunity. However, administration of unbound α-GalCer can drive iNKT cells into an anergic state (31-33). We adoptively transferred DCs loaded with both peptide antigen and α-GalCer (34), but found the subsequent T cell responses were low compared to responses seen after α-GalCer was given in association with whole protein or cell-associated protein (1, 35-37).

We then adoptively transferred α-GalCer-loaded tumor cells as a way of using cross-presentation of tumor antigen by host DCs in the presence of inflammatory mediators. In this study, we showed that i.v. injection of tumor/Gal resulted in IFN-γ secretion by iNKT and NK

cells (38). Several different tumor cell lines, when loaded with α-GalCer, failed to establish tumors upon i.v. injection even six months after vaccination, and this resistance was dependent on NK and iNKT cells. When we administered B16/Gal or CD1d highly-expressed variant B16/Gal ($CD1d^{hi}$B16/Gal), mice were protected from the development of B16 lung metastases due to the adjunctive effects of NK cells.

Mice given tumor/Gal intravenously developed innate iNKT cell-dependent immunity against subsequent i.v. tumor challenge, as expected. Surprisingly, these mice also became resistant to subcutaneous challenge with tumor cells, unlike mice given DC/Gal i.v. (37, 39). The $CD4^+$ and $CD8^+$ T cell-mediated resistance to tumor challenge was due to antigen uptake by host DCs and their subsequent maturation by activated iNKT cells. After tumor regression occurred, T cells responding to a variety of specific tumor antigens, such as Trp2, Tyrp, Dct and gp100, persisted as memory T cells. When glycolipid-loaded tumors are captured by DCs *in situ*, DCs perform two kinds of cross-presentation: the presentation of antigen peptide to $CD8^+$ T cells and the presentation of glycolipids to iNKT cells.

This combination of tumor-associated antigen and danger signal triggers DCs to initiate a strong adaptive immune response.

4. *IN VIVO* DC TARGETING THROUGH SPECIFIC RECEPTORS

DCs, generated *ex vivo* from monocytes or hematopoietic stem cells, have been the focus of many novel vaccine strategies over the past decade. These studies have to grapple with a number of variables, including methods of DC maturation and, when using DCs from patients with underlying clinical entities such as cancer or autoimmune diseases, the number and function of the generated DCs. In light of these drawbacks, a new approach is being developed in which antigens are directly targeted to DCs *in vivo*.

In order for this approach to be successful, the biological properties of DCs *in situ*, i.e. the efficiency of antigen uptake and the mechanisms of maturation, need to be better understood. Antigen should be targeted to DCs in conjunction with the maturation stimulus that will elicit the desired immune response.

DCs express a large collection of C-type lectins receptors (CLRs), such as the mannose receptor (MR), Langerin, DEC205 (CD205), DC-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN)/CD209, Dectin-1 and lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), which are molecules that bind the carbohydrate moiety of glycoproteins (40). CLRs act as anchors for a large number of microbes, including viruses, bacteria, parasites, and fungi, and allow their internalization.

One means to facilitate the uptake and processing of exogenous soluble antigen has been to deliver Ag via immune complexes directed at MHC class II and Fcγ RI present on antigen presenting cells (APCs). Strategies using CLR-specific antibody or ligand conjugated with antigens have been developed extensively by characterizing CLRs. Although FcR, MHC class II and DC-SIGN have been successfully targeted with model Ags, the path of antigen presentation in murine models appears to be predominantly MHC class II restricted.

4.1. Mannose receptor (MR)

The MR is a member of the calcium-dependent lectin receptor family, which has characteristic carbohydrate recognition domains with selective binding to specific glycans (40). MR is expressed by tissue macrophages (Mϕ) and lymphatic and hepatic endothelia in humans and mice. MR is also expressed by subsets of DCs, primarily interstitial DCs as well as DCs cultured from human monocytes and mouse bone marrow. The MR property to recycle constitutively allows a rapid and successive accumulation of its natural ligands or mannosylated Ag into intracellular compartments that may then access the processing pathways for MHC I and II presentation. In *in vitro* studies, the development of fusion proteins consisting of anti-human MR antibody (B11) and different tumor-associated Ags, such as hCG or pmel17 has resulted in targeted Ag delivery to APCs (41, 42). Tang *et al* developed a cancer immunotherapy in mice based on a mannan-mediated gene delivery system (43). They injected mice with DNA encoding ovalbumin (OVA) conjugated to oxidized or reduced mannan-poly-L-lysine. This induced both $CD8^+$ and $CD4^+$ T cell responses and antitumor immunity. Interestingly, this immune response was enhanced by concomitant immunization with a TLR agonist.

4.2. DEC205

DEC205 is a member of the CLR family and has a number of characteristics which make it an interesting prospect for *in vivo* antigen-targeting studies. DEC205 is expressed mainly on DCs in mice. In humans, DEC205 is expressed in its highest levels on mDCs, but can also be found on B cells, T cells, monocytes, macrophages and NK cells (40). DEC205 recycles through late endosomal or lysosomal compartments and mediates antigen presentation. Initial studies targeting antigen to DEC205 used antigen sequences fused at the carboxy terminus with the heavy chain of an anti-DEC205 mAb. These studies resulted in peripheral tolerance to the antigen due to the natural tendency of $DEC205^+$ DCs to induce tolerance under steady state conditions (44). Tolerance can be switched to immunity by administering antigen coupled with anti-DEC205 mAb together with anti-CD40 mAb.

Steinman *et al* established chemically coupled the protein OVA to the anti-DEC205 antibody or engineered the cDNA of the heavy chain of an anti-DEC205 to express sequences for antigenic peptides in frame at its carboxy terminus. They demonstrated the combination of DC targeting and a maturation stimulus improves OVA-specific $CD4^+$ and CD8 T^+ cell responses (2). DEC205 specific antibodies fused to protein antigen, peptide antigen, or protein antigen-loaded liposomes lead to antigen

presentation via MHC class I and II but need adjuvants, such as anti-CD40 antibodies and/or TLR ligands, to induce adaptive immunity. Using DEC205 mAb, vigorous $CD4^+$ and $CD8^+$ T cell responses have been generated to a variety of foreign proteins, including OVA, HIV gag, trp2, and malarial circumsporozoite protein (45-47). Attempts at inducing immunity to survivine have resulted in very weak responses in the $CD8^+$ T cell compartment (48). Perhaps this is due to the importance of antigen quantity or T cell antigen affinity in the generation of a $CD8^+$ T cell responses (2, 45, 47, 48). The unusually strong and protective $CD4^+$ T cell response seen after DEC-205-targeted vaccination makes this a promising area to explore for future vaccine strategies.

4.3. DC-SIGN

DC-SIGN is predominantly expressed on immature DCs and at lower levels on mature DCs and macrophages (40). Unfortunately, murine DC-SIGN is quite different from human DC-SIGN in both protein structure and patterns of expression and therefore cannot be used as a preclinical model. *In vitro* studies using a model antigen conjugated to humanized DC-SIGN-specific antibody successfully target human monocyte-derived DCs. This leads to the presentation of antigen by MHC class I and II molecules and induction of both naïve and memory T cell responses.

4.4. Others

Lox-1 mediates calcium-dependent recognition of phosphatidylserine and apoptotic cells (49). Some C-type lectins have signaling motifs in their cytoplasmic regions and deliver activation or suppression signals. Dectin-1, a β-glucan-specific CLR mediating the phagocytosis of yeast, delivers signals to produce IL-2 and IL-10, or IL-12 and TNF-α through distinct pathways (50).

5. *IN VIVO DC MATURATION LED BY DANGER SIGNALS*

DCs phagocytose dying cells *in situ*. Soon after ingestion, components of dying cells activate and enhance antigen presentation by DCs. These endogenous activating molecules are collectively called damage-associated molecular patterns (DAMPs) and include heat shock proteins (HSPs), high mobility group box 1 protein (HMGB1), β-defensin, uric acid, extracellular ATP and hyaluronan (51). DAMPs stimulate DCs through TLRs or NLRs (Figure 1).

5.1. HSP

Almost all cells contain HSP within their cytosols. Many different HSPs, including HSP70, grp96 and HSP90, induce DC maturation and act as adjuvants *in vivo*. DC maturation by HSPs is mediated through TLR 2, TLR4, CD40, CD91, CCR5, Lox-1 and SREC-1 (51). HSPs, such as gp96, HSP70 and HSP90, undergo receptor-mediated uptake by APCs with subsequent HSP-associated peptide presentation on MHC class I molecules (52-54). HSP70 and HSP90 activity occurs through two different pathways: either via the proteasome (TAP-dependent) or the endosome (TAP-independent). Bortezomib, a specific inhibitor of 26S proteasome, induces death in tumor cells expressing HSP90, and also demonstrates the clinical efficacy against several human tumors, including myeloma (55).

5.2. HMGB1

HMGB1, a cytokine-like factor released from dying cells, induces macrophages to secrete proinflammatory cytokines, such as TNF-α, IL-6, and HMGB 1 itself (56). Thus, HMGB1 represents an endogenous DAMP that triggers a cascade of inflammatory responses in the absence of microbes. HMGB1 binds to multiple receptors including TLR2, TLR4, and the receptor for advanced glycation and products (RAGE) expressed on macrophages, monocytes, DCs and NK cells. Some anti-cancer drugs lead to immunogeneic tumor death i.e. up-regulation of "eat me" signals and the release of DAMP (HMGB1) from dying cells (57). Apetoh *et al* recently showed that anthracyclins induce the rapid translocation of calreticulin to the cell surface and induce tumor antigen-specific T cell immunity via DC activation through HMGB-1-TLR4 signaling from dying tumor cells (58). To induce inflammatory cytokine production by murine and human DCs using rHMGB-1 in *in vitro* assays, the amount of rHMGB-1 added needs to be greater than 100 μg/ml (59, 60). However, HMGB-1 released from dying cells (10-200 ng/ml) does not achieve this level *in vivo* (58, 61). In light of this, HMGB-1 may need to act in concert with other stimuli, such as CpG, to stimulate DCs *in vivo* (62).

5.3. Uric acid

Soluble uric acid, a degradation product from purines, does not induce DC maturation, however crystals of monosodium urate (MSU) have a strong capacity to activate DCs in a TLR4-independent fashion (63). Recently, NALP3 has been identified as the receptor for MSU and triggers DCs to secrete proinflammatory cytokines, particularly IL-1β (64). Mammals have relatively high constitutive concentrations of uric acid in the blood (40-60 μg/ml). Uric acid becomes saturated and begins to precipitate *in vivo* at the concentration of 70 μg/ml (64). Because the cytosol contains high concentrations of uric acid (approximately 4 mg/ml, which increases even more when injured cells degrade their RNA and DNA), the local environment becomes super saturated with uric acid after a dying cell lyses, thus favoring the formation of MSU.

5.4. ATP

ATP is stored in the cytosol and accumulates in inflamed tissues. It is released into the extracellular space upon cell activation or from damaged and dying cells. Extracellular ATP serves as a danger signal to alert the immune system of tissue damage by acting on P2X or P2Y receptors (65). ATP regulates DC migration along lymph node-directing chemokines and inhibits TNF-α and IL-12p70 secretion and Th1 polarization.

5.5. Hyaluronan

Low molecular weight fragmentation products of the polysaccharide hyaluronic acid (sHA) released during inflammation have been shown to be potent activators of DCs and macrophages (66). It has recently been shown that hyaluronan fragments act as an endogenous danger signals by engaging TLR2 and TLR4 (67).

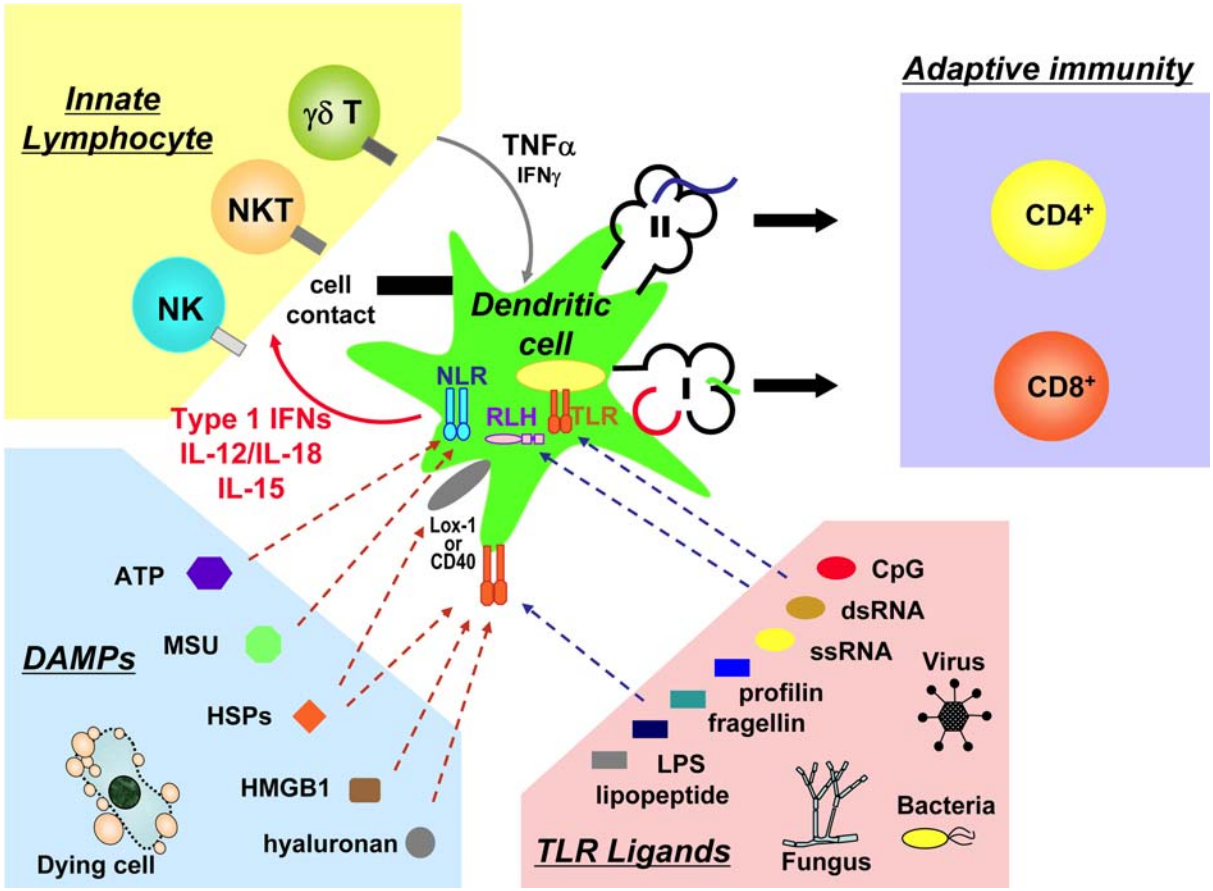

Figure 1. DC maturation is essential to the initiation of adaptive immunity. The three known groups of maturation stimuli are represented in the figure. First, a variety of ligands, such as LPS, CpG DNA and double stranded RNA, induce DC maturation through the Toll-like receptor (TLR) family or retinoic acid-inducible gene I (RIG-1)-like helicases (RLHs). Second, innate lymphocytes, such as NK cells, NKT cells and γδ T cells, become activated by ligands or antigens and induce DC maturation through cell-cell contact and cytokines like TNF-α and IFN-γ. Third, a group of damage-associated molecular patterns (DAMPs) can mature DCs through TLRs or Nod-like receptors (NLRs). (ATP; Adenosine triphosphate, MSU; monosodium urate, HSP; heat shock protein, HMGB1: high mobility group box 1)

6. PERSPECTIVE

Enhancing DC function through immunization has more clinical applications than the administration of inflammatory cytokines or antibodies alone. For example, harnessing DCs through the activation of innate immunity via the administration of whole tumor cells loaded with α-GalCer offers several advantages over these other methods (37, 38). Whole tumor cells deliver a wide range of peptides for DCs to process and present, leading to a broad T cell response to many different tumor antigens. α-GalCer activates iNKT cells leading to DC maturation. Importantly, maturing DCs *in situ* secrete cytokines and chemokines and express costimulatory molecules that are stimulatory to naïve T cells.

Therefore, as we discussed, approaches of combining DC-targeted antigen delivery with stimulators of the innate immune system most closely reproduces conditions seen *in vivo* and leads to a broader and more effective adaptive immune response.

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