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Harnessing the immune system to fight cancer with Toll-like receptor and RIG-I-like receptor agonists

Carole Bourquin[1,2,3], Aurélien Pommier[2], Christian Hotz[1, 4]

[1]Chair of Pharmacology, Faculty of Science, University of Fribourg, 1700 Fribourg, Switzerland

[2]School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 1211 Geneva, Switzerland

[3]Department of Anesthesiology, Pharmacology and Intensive Care, Faculty of Medicine, University of Geneva, 1211 Geneva, Switzerland

[4]Current address: BioNTech RNA Pharmaceuticals, 55131 Mainz, Germany

Correspondence: C. Bourquin, MD, PhD, E-mail: carole.bourquin@unige.ch

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Abstract

Cancer immunotherapy has come of age with the advent of immune checkpoint inhibitors. In this article we review how agonists for receptors of the innate immune system, the Toll-like receptors and the RIG-I-like receptors, impact anticancer immune responses. Treatment with these agonists enhances the activity of anticancer effector cells, such as cytotoxic T cells and NK cells, and at the same time blocks the activity of immunosuppressive cell types such as regulatory T cells and myeloid-derived suppressor cells. These compounds also impact the recruitment of immune cells to the tumor. The phenomena of pattern-recognition receptor tolerance and reprogramming and their implications for immunotherapy are discussed. Finally, novel delivery systems that target the immune-stimulating drugs to the tumor or the tumor-draining lymph nodes to enhance their efficacy and safety are presented.

The promise of cancer Immunotherapy

In cancer immunotherapy, the ultimate goal is to restore effective immune responses against malignant tumors. In 2018, James Allison and Tasuku Honjo were awarded the Nobel prize in Medicine for their work on immune checkpoints, which has led to an entirely new class of immunotherapeutic drugs, termed immune checkpoint inhibitors. These drugs have become standard-of-care in several types of cancer, and can bring lasting remissions or even cure up to 40% of patients with metastatic melanoma and 20% of patients with advanced lung cancer[1]. Immune checkpoints are negative regulators of immune activation that limit antitumor immune responses. The discovery that antibodies directed against these checkpoints could release these brakes has led to unprecedented numbers of treated patients with long-lasting antitumor immune responses. However, a measurable clinical response is observed only in a minority of patients[2]. One reason seems to be that many tumors lack the ability to recruit cytotoxic T cells, which are instrumental for the antitumor response[2]. Strategies that modulate the local tumor microenvironment and reinforce the migration of T cells into the tumor are therefore urgently needed to complement existing treatments. One emerging strategy to enhance the efficacy of checkpoint inhibitors is the combination with modulators of innate immunity, such as Toll-like receptor agonists[3,4].

Toll-like receptors and RIG-I-like receptors

Toll-like receptors (TLRs) belong to the larger group of pattern-recognition receptors which recognize conserved molecular patterns from microbial pathogens[5]. Their activation is the initial step in a cascade of events leading to stimulation of innate immunity, characterized by the secretion of pro-inflammatory cytokines and to adaptive immune responses. In addition to the membrane-bound Toll-like receptors, the cytoplasmic RIG-I-like receptors (RLRs) play a key role in the detection of microbial nucleic acids[6]. The responses induced by stimulation of these two receptor families range from the triggering of antiviral gene programs, including the production of type I interferon and of inflammatory cytokines, to the induction of apoptosis[5]. The nucleotide-sensing TLRs 3, 7/8 and 9, expressed mainly by immune cells, are localized in the endosome[5]. Whereas TLRs 7 and 8 are expressed in humans on many types of myeloid cells, TLR9 expression is limited to B lymphocytes and a subset of dendritic cells termed plasmacytoid dendritic cells[5]. These TLRs use two different signaling pathways to activate innate responses: The TLRs 7/8 and 9 signal via the adaptor molecule MyD88, leading to activation of NFkB and secretion of pro-inflammatory cytokines[7] (Figure 1). In addition, in the immune subset of plasmacytoid dendritic cells, ligation of TLR7 and 9 leads to secretion of type I interferon, also via the MyD88-dependent pathway (not depicted)[8]. TLR3, in contrast, utilizes the adaptor molecule TRIF to induce expression of type I interferon via the transcription

factor IRF3, as well as pro-inflammatory cytokines[9]. The cytoplasmic RLRs Melanoma Differentiation-Associated protein 5 (MDA-5) and retinoic acid-inducible gene I (Rig-I) are nearly ubiquitously expressed in immune and non-immune cells[5,8]. MDA-5 signals in a MyD88-independent manner and its activation induces the production of high amounts of type I interferon by all cells in the body[10]. The important immune functions of TLRs and RLRs have raised hopes that controlled pharmacological activation of these receptors may induce effective anticancer immune responses.

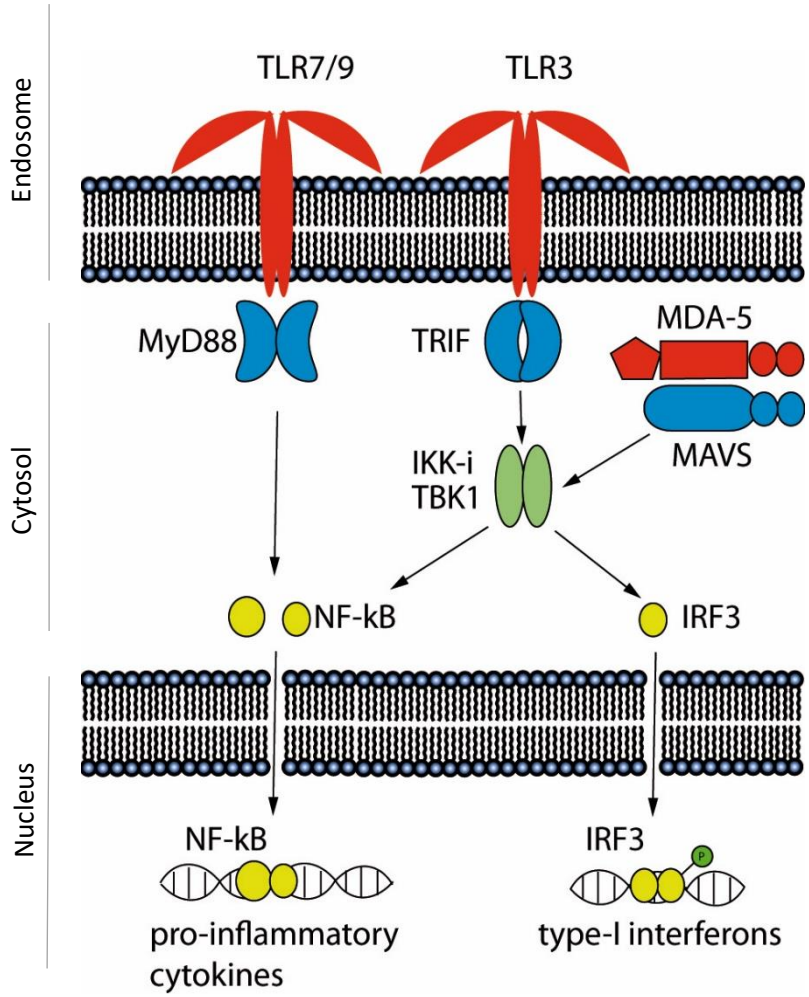

Figure 1: Overview of pattern-recognition receptor pathways

Signaling of nucleotide-sensing receptors localized to the endosome or the cytoplasm leads to transcription of genes for pro-inflammatory cytokines and type-I interferons. Red, receptors; blue, adaptor proteins; green, kinases; yellow, transcription factors.

The therapeutic profile of TLR and RLR agonists

One TLR agonist that has been applied successfully in the clinic for more than a decade is imiquimod, a small molecule immune response modifier targeting TLR7, which is used in a cream for the treatment of basal cell carcinoma and other malignancies in the skin[11]. In addition, several TLR and RLR agonists have been assessed in clinical trials for the treatment of non-skin cancer. Table 1 lists completed and prematurely terminated clinical trials for TLR7/8/9 and RIG-I agonists administered internally, with the exclusion of studies in which these agonists were used as vaccine adjuvants. Table 2 lists ongoing clinical trials.

Toll-like receptor 9 agonists, which were the first to be applied systemically in the clinic, are generally oligonucleotides containing specific palindromic CpG motifs[5]. Agatolimod (also known as ODN 2006, CpG 7909, PF-3512676, VaxImmune, and ProMuneT) is a well-characterized CpG oligodeoxynucleotide and TLR9 agonist that has been studied either as single agent or in combination with established therapies. With the exception of T-cell lymphoma, where objective clinical responses were observed (NCT00043420)[12], little or no clinical benefit was seen in the majority of studies (Table 1)[13,14]. Agatolimod was generally well tolerated, with the most common adverse events being mild to moderate systemic flu-like symptoms, grade 3/4 neutropenia and thrombopenia (NCT00040950, NCT00185965, NCT00438880). Its clinical development was nevertheless discontinued after it increased toxicity without improving outcomes in advanced non-small cell lung cancer[13,14].

SD-101 is another CpG oligonucleotide acting as TLR9 agonist. In preclinical studies, it was established that this compound needed to be injected intratumorally for efficacy[3]. When tested in combination with radiotherapy for B-cell lymphoma, patients not only showed tumor reduction at treated sites, but also at untreated sites, indicating the development of systemic immunity (NCT02266147)[15]. Combination of SD-101 with an immune checkpoint inhibitor for the treatment of metastatic melanoma was well tolerated and also resulted in antitumor immune responses at distant, non-injected sites (NCT02521870)[3]. Several clinical studies with SD-101 administered intratumorally in combination with checkpoint inhibitors or targeted therapies are ongoing (Table 2).

In contrast to TLR9 agonists, TLR7/8 agonists are generally low molecular weight compounds. Since Toll-like receptors 7 and 8 have a wider distribution than TLR9 in humans, this is expected to lead to a different type and strength of immune responses for TLR7/8 agonists[5]. The very different pharmacokinetics of small molecules vs. oligonucleotides may also impact the therapeutic profiles. The systemic application of first-generation TLR7/8 agonists such as 852A was unsuccessful due to limited efficacy and severe adverse effects, such as neutropenia, dehydration, and unexpected cardiotoxicity[16–18]. Second-generation TLR7/8 agonists are currently undergoing clinical testing (Table 2).

RIG-I agonists are synthetic RNA oligonucleotides with specific phosphorylation patterns that have shown potent antitumoral effects in preclinical studies[19–24]. Interestingly, these agonists not only stimulate anticancer immune responses, but also have a pro-apoptotic effect in cancer cells[19]. The synthetic oligonucleotide RGT100, a RIG-I agonist, was tested in a phase I/II clinical trial in advanced tumors (NCT03065023), but the results were to date not reported (Table 1). Activation of MDA5 by poly(I:C), a long double-stranded synthetic RNA, induced apoptosis in cancer cells and stimulated anticancer immune responses in a preclinical model of pancreatic cancer[25,26]. In humans, poly ICLC (Hiltonol®) was mainly studied as an adjuvant for cancer vaccines[4].

Table 1. Principal clinical trials completed or terminated to investigate the therapeutic profile of TLR7/8/9 and RLR agonists in cancer patients.*

Status	Target	Molecule	Indication	Phase	Route	Notes	Ref.
Completed	TLR7	Imiquimod	Head and neck	II	SC	In combination with cetuximab vs cetuximab alone	NCT01040832 [18]
		852A	Breast, ovarian, endometrial, cervical	II	SC	Single agent	NCT00319748 [17]
			Melanoma	II	IV	Single agent	NCT00189332 [16]
	TLR9	SD-101	Lymphoma	I/II	IT	In combination with ipilimumab and RT	NCT02254772
			Low-grade B-cell lymphomas	I/II	IT	In combination with RT	NCT02266147 [15]
		Agatolimod	Low-grade B-cell lymphomas	II	IT	In combination with RT	NCT00880581
			Advanced non small cell lung	II	SC	In combination with erlotinib vs erlotinib alone	NCT00321815
			Non-Hodgkin lymphoma	I/II	IV	In combination with rituximab, and yttrium Y 90 ibritumomab tiuxetan	NCT00438880
			Chronic lymphocytic leukemia	I	IV/SC	Single agent	NCT00233506
			Non-Hodgkin lymphoma	I/II	IT/PT	In combination with RT	NCT00185965
			T-Cell Lymphoma	I/II	SC	Single agent	NCT00043420 [12]
			Renal	I/II	SC	Single agent	NCT00043407
			Non-Hodgkin Lymphoma	I	IV/SC	In combination with rituximab	NCT00040950
			Carcinoma, metastatic breast	I/II	IV	In combination with herceptin	NCT00031278
		MGN1703	Advanced colorectal	II	SC	In combination with chemotherapy bevacizumab vs chemotherapy bevacizumab	NCT01208194
Terminated	RIG-I	RGT100	Advanced or recurrent tumors	I/II	IT	Single agent	NCT03065023
	TLR7/8	MEDI9197	Solid tumors	I	IT	Single agent and in combination with durvalumab and/or RT	NCT02556463

		852A	Hematologic malignancies	II	SC	Single agent	NCT00276159
	TLR8	Motolimod	Solid tumors	Ib	SC	In combination with cyclophosphamide	NCT02650635
	TLR9	Agatolimod	Advanced non small cell lung	III	SC	In combination with paclitaxel/carboplatin vs paclitaxel/carboplatin alone	NCT00254891 [14]
				III	SC	In combo with gemcitabine/cisplatin vs gemcitabine/cisplatin alone	NCT00254904 [13]
				II	Unknown	In combination with pemetrexed vs permetrexed alone	NCT00321308
			Advanced or metastatic breast	II	SC	In combination with trastuzumab	NCT00824733
		SD-101	Advanced solid malignancies	I/Ib	IT	In combination with IL-10 agents	NCT02731742
			Lymphoma	I	IT	In combination with RT after allogenic hematopoietic cell transplantation	NCT01745354
		IMO-2055	Head and Neck	Ib	SC	In combination with 5-FU + Cisplatin + cetuximab	NCT01360827
			Colorectal	I	SC	In combination with FOLFIRI + cetuximab	NCT00719199

Abbreviations: SC, Subcutaneous; IT, Intratumoral; IV, Intravenous; RT, Radiotherapy.

* Clinical trials using TLR agonists as adjuvant for cancer vaccination or topical administration were not included

Table 2. Principal clinical trials to investigate the therapeutic profile of TLR7 and TLR9 agonists in cancer patients.*

Status	Target	Molecule	Indication	Phase	Route	Notes	Ref.
Recruiting	TLR7	NJH395	NON-breast HER2+	I	IV	Immune stimulator antibody conjugate targets HER2	NCT03696771
		NKTR-262	Solid tumors	I/II	IT	In combination with CD122-biased agonist and nivolumab	NCT03435640
		DSP-0509	Neoplasms	I	IV	Single agent	NCT03416335
		LHC165	Solid tumors	I	IT	In combination with PRD001 (anti PD-1)	NCT03301896
	TLR9	MGN1703	Solid tumors	I	SC	In combination with ipilimumab	NCT02668770
		IMO-2125	Metastatic melanoma	I/II	IT	In Combination With Ipilimumab or pembrolizumab	NCT02644967
			Refractory melanoma	III	IT	In combination with ipilimumab	NCT03445533
		CMP-001	Melanoma	I	IT	In combination with pembrolizumab	NCT02680184
			Melanoma	II	IT	In combination with nivolumab	NCT03618641
			Metastatic colorectal	I	IT/SC	In combination with combined immunotherapy and radiosurgery	NCT03507699
		SD-101	Lymphoma	I	IT	In combination with an anti-OX40 antibody, BMS-986178 and RT	NCT03410901
			Solid tumors & lymphoma	I/II	IT	In combination with epacadostat and RT	NCT03322384
			Prostate carcinoma	II	IT	In combination with pembrolizumab and RT	NCT03007732
			Follicular lymphoma	Ib/II	IT	In combination with ibrutinib and RT	NCT02927964
			Solid tumors	Ib/II	IT	In combination with pembrolizumab	NCT02521870 [(3)]
Not yet recruiting			Solid tumors	I	IT	In combination with Anti-OX40 Antibody BMS 986178	NCT03831295

Abbreviations: SC, Subcutaneous; IT, Intratumoral; IV, intravenous; RT, Radiotherapy.

* Clinical trials using TLR agonists as adjuvant for cancer vaccination or topical administration were not included

How TLR and RLR agonists impact the anticancer immune response

The clinical success of TLR and RLR agonists is based on their capacity to efficiently mobilise both innate and adaptive immunity. Indeed, RNA-based compounds stimulating TLR7 or MDA-5 efficiently activate antitumoral $CD8^+$ cytotoxic T cells[27,28] and promote strong type I interferon responses[29]. In addition to $CD8^+$ T cells, NK cells play a pivotal role in anti-tumor immunity. It was shown that TLR7 activation via RNA-based motifs leads to effective treatment of NK-sensitive tumors[30,31]. CD8 and NK priming is achieved indirectly through the activation of dendritic cells, which, when stimulated by TLR7 agonists, produce the antitumoral cytokines IL-12 and IFNα. Thus, TLR7 agonists activate both cytotoxic T cells and NK cells, which are the two main effector cell types for the antitumoral immune response. The effects of TLR and RLR agonists on immune cell subtypes is depicted in Figure 2.

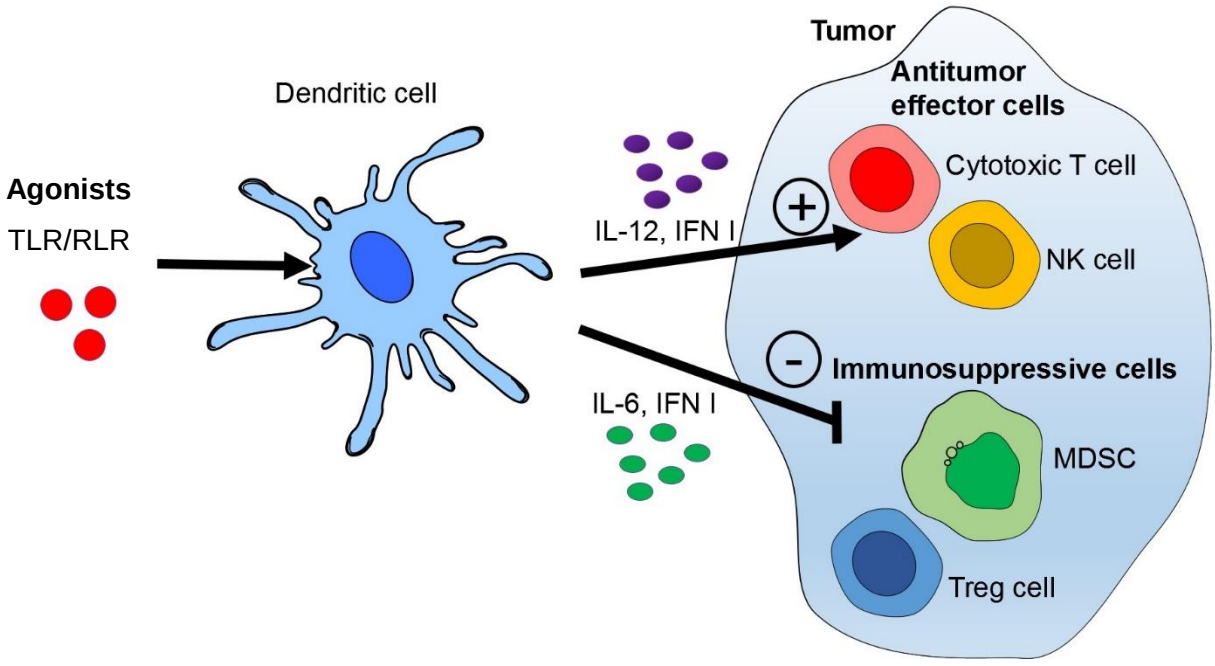

Figure 2: Impact of TLR and RLR agonists on tumor-infiltrating immune cells. TLR and RLR agonists activate dendritic cells to produce pro-inflammatory cytokines. These cytokines activate effector cells, namely cytotoxic T cells and NK cells enhancing their antitumor activity. At the same time, the immunosuppressive function of Treg cells and myeloid-derived suppressor cells (MDSC) is

blocked, and the recruitment of these cells to the tumor is prevented. IL-6: interleukin 6; IL-12, interleukin 12; IFN I, type I interferon; NK cell, natural killer cell; Treg, regulatory T cell; MDSC: myeloid-derived suppressor cell.

In addition, TLR7 and TLR9 agonists inhibit the function of suppressive cells of the immune system. Regulatory T cells play an essential role in the maintenance of immune homeostasis. In cancer, these cells contribute to tumor-associated immune suppression and their presence within the tumor is predictive for poor prognosis[32]. It has been demonstrated that TLR7 and TLR9 agonists inhibit the suppressive function of regulatory T cells[33]. This effect was entirely mediated by dendritic cells, via secretion of the cytokine IL-6 (Figure 2). TLR activation not only affects the function of regulatory T cells, but also inhibits their recruitment to the tumor. Indeed, numerous types of tumors secrete the chemokine CCL22, which attracts regulatory T cells[34]. Treatment with TLR7 or TLR9 agonists suppresses the intratumoral production of CCL22 and this prevents the recruitment of regulatory T cells to the tumor[35]. The block in CCL22 secretion is dependent on the production of type I interferon and is an essential step in the inhibition of cancer progression by TLR and RLR agonists[35].

Myeloid-derived suppressor cells (MDSC) are another subset of immunosuppressive cells. These cells accumulate both systemically and in the tumor microenvironment because of a maturation block which prevents their differentiation. They can infiltrate tumors and contribute to tumor-induced immune suppression[32]. TLR7 and TLR9 agonists can block their suppressive activity by promoting their differentiation and restoring the balance of mature to immature myeloid cells[36,37] (Figure 2). Indeed, after treatment with a TLR agonist, MDSC mature into antigen-presenting cells with the capacity to induce rather than suppress antigen-specific T-cell responses[37]. Importantly, this effect is also mediated through the production of type I interferon[36]. Systemic application of the small molecule TLR7 agonist resiquimod also affects MDSC numbers and migration patterns, as this leads to a reduction in both circulating and intratumoral MDSC[37,38]. Thus, the anticancer activity of TLR7 and TLR9 agonists is due to a block of the suppressive function of regulatory T cells and MDSC on one hand and the prevention of their recruitment to the tumor on the other hand.

Toll-like receptor tolerance: an obstacle for immunotherapy

Receptor stimulation and cytokine secretion are usually tightly regulated. However, repeated stimulation of pattern-recognition receptors leads to tolerance, preventing further cytokine secretion. This phenomenon was initially termed “endotoxin tolerance”, meaning that mice prestimulated with lipopolysaccharides (LPS), which are recognized by TLR4, are resistant to

further stimulation with LPS[39]. Tolerance is not limited to TLR4, but also occurs when repeatedly stimulating the TLRs 4, 5 and 7 and 9[40]. TLR stimulation not only results in "homotolerance", defined as tolerance towards a second stimulation via the same receptor, but also induces "heterotolerance" towards other TLRs[41]. Of note, the induction of heterotolerance depends on the signaling pathways: It has been shown that MyD88-dependent stimuli render only MyD88-dependent pathways tolerant but not MyD88-independent ones[42].

TLR tolerance has been shown to reduce the efficacy of TLR7 agonists for the immunotherapy of cancer[41]. In mice, a single injection of the TLR7 agonist resiquimod leads to tolerance towards a second stimulation beginning 24 h after injection and lasting for up to five days. Thus, the repeated administration of TLR7 ligands can lead to low efficacy in cancer therapy. Of note, protocols used in clinical trials investigating the therapeutic potential of systemic TLR7 stimulation in cancer have relied on single injections given every two to three days[43]. In mice, this schedule would result in tolerance and might be the reason for the limited success with this protocol in clinical studies[43]. To better circumvent tolerance, a protocol of fractionated stimulation with resiquimod in cycles separated by 5-day intervals was designed. The use of this protocol in a murine cancer model led to an efficient block of tumor growth and was more efficacious than the schedule used in clinical studies, although the cumulated dose was lower [41].

Enhancing the TLR7 response by receptor reprogramming: Timing is everything

One strategy to enhance the efficacy of treatment with pattern-recognition receptor agonists may be to use sequential applications of MyD88-independent and MyD88-dependent stimuli in order to "prime" the immune response. Based on an extensive screening of molecular signaling pathways, it was determined that pretreatment with MyD88-independent RLR and TLR agonists, such as poly(I:C), reprograms all dendritic cells in the body, profoundly modifying their response to subsequent stimulation[44]. Reprogrammed dendritic cells become more sensitive to MyD88-dependent stimuli, such as TLR7 and TLR9 agonists, and responses to these stimuli are enhanced (Figure 3). At the same time, the dendritic cells do not respond any more to MyD88-independent stimuli[44]. The reprogramming occurs within 24 h of the first compound application, and is dependent on type I interferon. At the pathway level, activation of the transcription factor IRF3 has been shown to be decreased following receptor reprogramming, whereas phosphorylation of TBK-1 was not altered[44]. This suggests that the reprogramming occurs at the level of IRF3. These findings contribute to the understanding of how signals from different classes of pattern-recognition receptors are integrated to program immune responses.

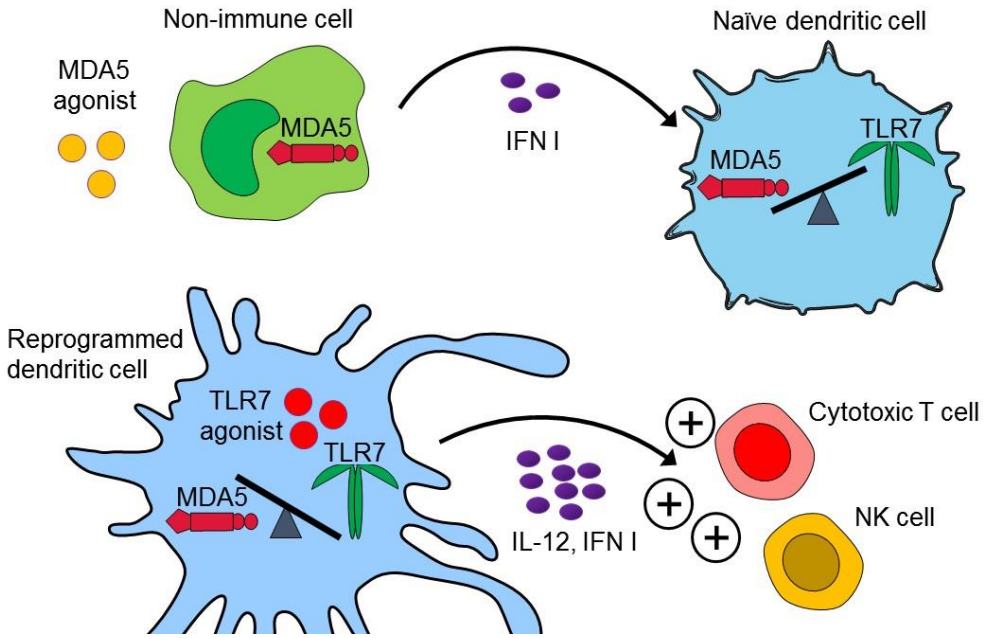

Figure 3: Pattern-recognition receptor reprogramming. Upon cytoplasmic activation of MDA5, non-immune cells produce type I IFN. This leads to receptor reprogramming of all dendritic cells in the body. Within 24 h, the response of dendritic cells to MDA5 is blocked and, at the same time, they become more sensitive to activation by TLR7 agonists. The production of IL-12 and IFN I is enhanced, leading to stronger cytotoxic T cell and NK cell responses. IL-12, interleukin 12; IFN I, type I interferon; NK cell, natural killer cell.

These rapidly induced and global changes in sensitivities of pattern recognition receptors were termed *PRR reprogramming*. This form of receptor crosstalk needs to be differentiated from simple synergies between separate receptor pathways due to kinetics, taking up to 24 h to come into effect[44]. It is tempting to speculate that PRR reprogramming differs from a phenomenon termed “trained immunity”, a type of long-lasting innate immune memory induced by a range of microbial constituents, vaccines or pathogenic conditions[45]. Indeed, the group of Medhzitov showed that LPS, which is known to induce tolerance shortly after an initial stimulation, facilitates long-term changes in innate cells by epigenetic remodelling[46]. It remains to be determined whether PRR reprogramming is mediated by chromatin modifications or long-lasting changes of metabolic states, which can be considered a feature of trained immunity.

To take advantage of receptor reprogramming, a sequential treatment with RLR and TLR agonists can prevent unresponsiveness to immune stimulation and lead instead to enhanced immune responses. Indeed, when poly(I:C) and resiquimod, two response modifiers used in

clinical trials, were sequentially administered, increased levels of the anti-tumor cytokines IFN-α and IL-12p70 were found[44]. This treatment also led to enhanced activation of cytotoxic T cells and differentiation of T helper cells, two cell types of the adaptive immune system involved in fighting cancer[47]. This strategy therefore allowed to overcome TLR tolerance and strengthen TLR7-dependent antitumor immune responses. Thus, the precise timing of immunotherapeutic protocols based on results from molecular and cellular studies is important to improve the efficacy of cancer treatments.

Novel drug delivery systems for TLR and RLR agonists

Important bioengineering advances have been made in order to improve the efficacy and safety of TLR and RLR agonists for cancer immunotherapy. For instance, the use of virus-like nanoparticles to deliver a TLR9 agonist in melanoma patients increased antitumor immune responses[48]. A local immune reaction was observed in the lymph nodes draining the injection sites. In fact, one goal of drug delivery systems is to target the immune-activating drugs to the site of induction of an immune response, such as the tumor-draining lymph nodes[49]. In addition, due to their phagocytic nature, dendritic cells can be selectively targeted by particulate delivery systems. Thus, TLR and RLR agonists can be delivered directly to dendritic cells by nanoparticles prepared from inorganic materials such as gold[50] or silica[51,52], or by biodegradable particles from e.g. gelatin[53–55], spider silk[56] or polymers[49,57]. The use of RNA-lipoplexes, where the RNA functions as a TLR7 agonist, has led to systemic targeting of dendritic cells, induction of IFN-I and potent induction of tumor-specific T cells in patients[58]. Delivery of a TLR7 agonist by nanoparticles can also enhance the response to immune checkpoint inhibitors[59]. Future bioengineering challenges in this field will be to improve loading efficiency of the drug, as well as the efficiency of delivery of the cargo to the site of action. Indeed, a large literature review concluded that only 0.7% of injected nanoparticles actually reach the tumor[60].

One compound of particular interest for loading onto a delivery system is resiquimod. As a small hydrophobic molecule, it presents interesting physicochemical properties and is certainly easier to load onto different types of nanoparticles than the nucleic acid-based TLR and RLR agonists. A delivery system composed of gold nanoparticles of 5 nm in diameter coated with an amphiphilic shell was developed, which allowed loading of resiquimod through non-specific adsorption. After subcutaneous injection of these resiquimod-loaded particles into tumor-bearing mice, they accumulated in the tumor-draining lymph nodes where they led to activation of the dendritic cells *in situ* causing a cytotoxic T-cell response[61]. This treatment completely blocked the growth of the large tumor and extended the survival of the mice much more than the free drug[61]. Thus, the delivery of resiquimod to the tumor-draining lymph node, which is

the site of initiation of the immune response against the tumor, was highly effective for triggering an anti-tumor response. In addition, the response was clearly systemic, since tumor-specific cytotoxic T cells were detected in the spleen, distant from the site of injection.

Thus, the development of drug transport systems that mediate the delivery of immune stimulators directly to their site of action, be it the tumor itself, a metastasis thereof or the tumor-draining lymph nodes, will be crucial in the future for *in situ* applications of small-molecule compounds that would normally spread non-selectively throughout the organism.

Conclusions

Safer and more effective TLR and RLR agonists have been developed in recent years for use in cancer immunotherapy. New ligands for pattern-recognition receptors have been identified, their mode of action has been characterized at the molecular and cellular levels, optimal dosage regimens have been defined and new principles for drug delivery systems have been designed. It is probable that the *in situ* application of TLR and RLR agonists or their targeted delivery will play an important role for the improvement of the response to immune checkpoint inhibitors in the coming years. Major challenges in bioengineering include the development of nanocarriers that can protect biological ligands such as oligonucleotides from degradation, improve access of TLR and RLR agonists to their intracellular receptors, and facilitate their targeting to dendritic cells. In addition, nanocarriers can restrict the systemic diffusion of the immunomodulatory drugs, thus reducing generalized immune activation and improving the safety of TLR and RLR agonists for cancer immunotherapy. Finally, controlled release of the immunomodulators by smart delivery systems may help to prevent TLR tolerance.

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