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Current and future drugs targeting one class of innate immunity receptors: the Toll-like receptors

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Innate immunity receptors are germline-encoded receptors that can sense molecular signatures of pathogens and cancer cells. Recent advances in immunology demonstrate the key role of these receptors in inflammation and initiation of subsequent immune responses, including adaptive immunity. Pharmaceutical interest in this field has grown with the retrospective demonstration that some marketed drugs targeting cancer or infectious diseases act via those receptors. In this review, I present an update on the scientific rationale for targeting one class of innate immunity receptor, the Toll-like receptors, and an update on the development status of corresponding drug candidates in infectious diseases, cancer, allergy and vaccines.

Introduction

The mammalian immune system protects against invading pathogens and cancer cells and can be divided into two major components: the innate immune response and the adaptive immune response. The former is composed of several types of cells, including dendritic cells (DCs), macrophages and monocytes, polynuclear cells (e.g. neutrophils and mast cells), natural killer (NK) cells, γδ T cells and natural killer T cells (NKT cells). The innate immune system detects large classes of pathogens or abnormal cells through a limited number of germline-encoded receptors such as Toll-like receptors (TLRs), and serves as a first line of defense against infectious agents, preventing rapidly dividing pathogens from overwhelming the infected organism.

The adaptive immune response is the basis of immunological memory, used by vaccines. It is mediated through T cells and B cells bearing clonal receptors (i.e. T-cell receptors and antibodies) generated randomly through somatic recombination. The T and B cells that express receptors with high affinity and specificity for molecular structures of pathogens or abnormal cells undergo clonal expansion, exert their effector function and are conserved as memory cells.

Interest in innate immunity cells and receptors has recently increased because their central role in triggering inflammatory signals that are necessary for a subsequent immune response cascade was demonstrated, and because it was found recently that innate immunity signals serve as gatekeepers for mounting an efficient adaptive immune response. Thus, coordinated actions of innate and adaptive immune cells will eventually lead to the complete removal of the pathogen and to the generation of memory, which will guarantee a more rapid and accurate response in case of re-infection (Table 1; Figure 1).

The structures recognized by innate immunity cells are a limited array of conserved molecules that represent the molecular signature of pathogens and transformed cells. These structures, usually called pathogen-associated molecular patterns (PAMPs), are highly conserved (e.g. bacterial cell wall components) and are often essential for pathogen survival.

Innate immunity receptors, referred to as pattern recognition receptors (PRRs), are a class of proteins capable of recognizing PAMPs. The recently discovered TLRs represent the best-characterized class of PRR. Ten different TLRs have been identified, each of them recognizing one, or several, PAMP. Signaling through PRRs is transmitted through evolutionary conserved inflammation pathways, for example via nuclear factor-κB (NF-κB) and activation of interferon regulatory factor (IRF), explaining the central role of PRRs in triggering inflammation processes that further enhance activation of innate immune effectors, and are necessary for the development of potent adaptive responses.

Genetic PRR defects lead to increased risks for different types of infections and cancer [1]. Agonists of PRRs are, therefore, attractive

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TABLE 1

Innate and adaptive immunity features

	Innate immunity	Adaptive immunity
Cells involved	Dendritic cells Monocytes and macrophages Mast cells Specialized lymphocytes (NK, γδ and NKT cells) Epithelial cells	B cells (humoral immunity) CD4 T cells (helper T cells) CD8 T cells (cytotoxic T cells)
Distribution of receptor for antigen	Broad expression and distribution on specialized cell subset	Clonally distributed
Receptor specificity	Broad (can recognize large groups of pathogens or abnormal cells) Pathogen-associated molecular pattern Stress-induced self Missing self (MHC down modulation in cancer)	Narrow (very specific of a particular pathogen or abnormal cell) Epitope (fraction of antigen recognized by antibodies) Antigenic peptides presented by MHC (recognized by TCR)
Kinetics of response	Immediate, short-lived	7–10 days to develop, can be lifelong (immunological memory)
Type of response	Triggering of inflammation Immediate effector function (macrophages, NK, γδ and NK T cells) Gatekeeper of adaptive immunity (regulatory function)	Effector function (humoral and cellular) Regulatory function (can induce sustained innate immunity response) Generation of memory cells (quicker response if same pathogen or abnormal cells is re-encountered)

targets to stimulate both arms of the immune response in infectious diseases and cancer indications; conversely, antagonists can be important in controlling some chronic inflammation processes. The natural PRR ligands, or PAMPs, are often the basis for first generation agonist molecules that can be developed for proof-of-concept experiments in preclinical models or in early clinical trials. This strategy has been applied to several TLRs, and a few other innate immunity receptors such as receptors of γδ T cells

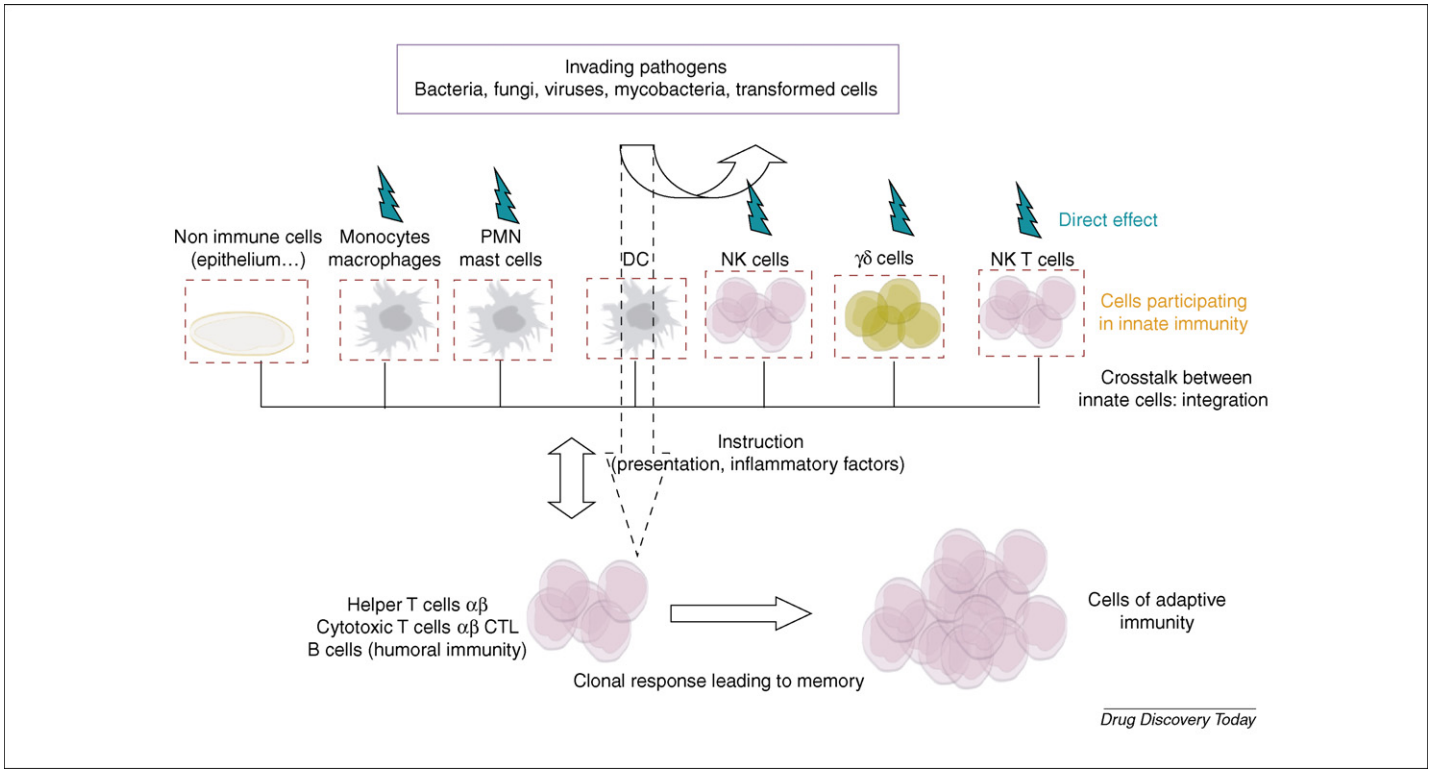

FIGURE 1

Innate immunity constitutes a first line of defense against pathogens or transformed cells. Innate immunity cells can detect conserved molecular signatures of pathogens. The innate immunity cells have direct, immediate effector function (lightning arrow) against pathogens, infected cells or abnormal cells, and regulatory functions. The different cells interact with each other to integrate the signal and instruct the adaptive immunity by cell–cell contacts and soluble mediators (dotted arrow) to generate specific effectors and memory (B cells, CD4 T cells and CD8 T cells). Adaptive immune cells (B cells and T cells) undergo clonal expansion and, in turn, regulate the innate immune response positively or negatively (double-headed arrow).
Abbreviations: DC, dendritic cell; PMN, polymorphonuclear cells; NK, natural killer cells; NKT, natural killer T cells; CTL, cytotoxic T lymphocyte.

[2,3] and NK cells [4,5]. Because drugs targeting TLRs are much more advanced in clinical development, this review will focus on this family of receptors, describe the current understanding of TLR function and the scientific rationale for developing TLR agonists, as well as the current status of drugs that target these receptors in vaccines, infectious disease, allergy and cancer.

Toll-like receptors: key receptors for inflammatory processes

Only six years have passed since the discovery of the first TLR [6–8], and rarely has a research field exploded so rapidly. All the TLRs (i.e. ten human TLRs) have now been cloned, a lot of their ligands discovered and the main signaling pathways identified – following homology searches in the human genome, the list seems to be closed. Many excellent in-depth reviews have been published describing the natural ligands and molecular pathways of signal transduction [9–11] and they are briefly reviewed here. TLR molecules are type I proteins with a conserved intracellular motif [e.g. Toll interleukin-1 receptor (TIR) domain – responsible for initiation of transduction] and an extracellular domain containing a leucine-rich repeat (LRR), thought to be responsible for ligand binding. Every TLR recognizes one or more PAMP. TLR2, either alone or in association with TLR1 and TLR6, recognizes cell wall components of bacteria, mycobacteria and fungi. TLR4 has specificity to lipopolysaccharide (LPS). TLR5 is specific for flagellin, a key organelle of motile bacteria. TLR3 is specific for double-stranded (ds)RNA, whereas TLR7 and TLR8 are specific for single-stranded (ss)RNA, both types of RNA representing signatures of different viruses. TLR9 recognizes DNA oligonucleotides containing an unmethylated CpG motif, the absence of methylation in CG being a signature of bacterial and some viral DNA.

The tissue distribution of TLRs is coherent with their role as sensors for innate immunity because they are expressed on innate immunity cells (e.g. DCs) and some epithelial tissues.

Although TLR expression is not restricted to DCs, TLR function has been studied extensively in this cell type because TLRs are essential mediators of DC activation and maturation into potent antigen-presenting cells (APCs). Several types of DC have been isolated and a considerable amount of literature exists on the subject, reviewed in Ref. [12]. My review will refer to the two main types of DC defined in humans and mice: (i) myeloid DCs (mDCs), prone to producing low amounts of type I interferon (IFN) and high amounts of inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-12 (IL12); and (ii) plasmacytoid DCs (pDCs), one of the main sources of type I IFN, producing low amounts of TNF-α and IL12. TLR1, 2, 4, 5, 6 and 10 are located at the cell surface of APCs (e.g. mDCs and macrophages) and are routed to phagosomes upon activation. TLR3, 7, 8 and 9 are seldom present at the cell surface and are preferentially expressed on endoplasmic reticulum where pathogen RNA and DNA can be routed. TLR3 and TLR8 are present on mDCs and macrophages in humans, and further expression of TLR3 on epithelial cells and NK cells has been described. TLR7 and TLR9 have been described on pDCs, and not mDCs, in humans, with further expression of TLR9 on B cells and NK cells. There is still some debate about the expression of TLRs on other cell types such as mastocytes and basophils. Finally, tissue distribution of certain TLRs varies in different species. The most striking cases include TLR8, which might be nonfunctional, in mice and the expression of TLR9 in mDCs and pDCs in mice; TLR9 is only expressed in pDCs in humans. TLR11 has been found to be inactive in humans, but is active in mice. Such differences must clearly incite caution when interpreting preclinical toxicity and efficacy data, and warrant the use of non-human primate models, where TLR distribution is closely related to humans; at least in the cases mentioned here.

All TLRs signal through a few adaptor molecules [i.e. myeloid differentiation factor 88 (MyD88), Toll receptor-associated activator of interferon (TRIF), MyD88-adaptor-like (MAL) and Toll receptor-associated molecule (TRAM), relayed to the inflammatory pathways involving NF-κB, Janus kinase (JNK)/p38 kinase and IFN regulatory factor (IRF)3, 5 and 7] [10,11] (Figure 2). TLR signaling induces the activation and maturation of APCs together with the production of inflammatory cytokines [e.g. type I IFN, inducible IFN protein 10 (IP10) – mostly from pDCs via TLR7, TLR9 and TNF-α, IL12 – mostly from mDCs and macrophages via TLR1, 2, 5, 6, 8 and possibly 10]. All these cytokines mainly enhance T helper type I (TH1) adaptive immune responses.

Synthetic agonists have been generated for at least some of the TLRs, and often consist of molecular mimics of the natural ligand. Many of them have been used in early-phase clinical trials.

Clinical trials involving Toll-like receptor agonists

The rationale for using TLR agonists that trigger inflammation will result in the production of cytokines, activation of innate immunity effector cells and, eventually, the adaptive immune response against cancer cells or infectious agents that could lead to long-term protection. Depending on the type of inflammatory signals they induce, different agonists targeting different receptors have been developed as vaccine adjuvants, anti-infectious agents, anticancer agents and in anti-allergy strategy. Several clinical trials leading, in some cases, to approved drugs have been performed in these different therapeutic fields and are reviewed in Table 2.

Vaccines

It is now apparent that changing from live attenuated or dead vaccines to more-defined antigens for vaccine preparation occurred at the expense of a great loss in immunogenecity, precisely because of the lack of innate immunity signals to initiate the adaptive immune response. Innate immunity receptor agonists therefore had a natural use as adjuvants of these new vaccines. Indeed, most TLR agonists are good candidates to enhance TH1 type responses because they activate type I IFN and the NF-κB pathway, inducing inflammatory cytokines such as TNF-α and IL12.

Some of these compounds are already present as adjuvants in commercial vaccines. Monophosphoryl lipid-A (MPL; an analog targeting TLR4 [13,14]) is part of the hepatitis B virus (HBV) vaccine Fendrix®, and has been approved for high-risk patients. MPL is also efficacious in vaccines against papillomavirus and genital herpes [15,16]. This adjuvant, and some derivatives, is now used extensively in clinical trials of prophylactic (e.g. malaria and tuberculosis) and therapeutic (e.g. cancer) vaccines [13].

TLR9 agonists are also in clinical development as adjuvants. These agonists resemble bacterial DNA and consist of short oligonucleotides bearing an unmethylated CpG motif with a phosphorothioate backbone to increase *in vivo* stability. Of note, several

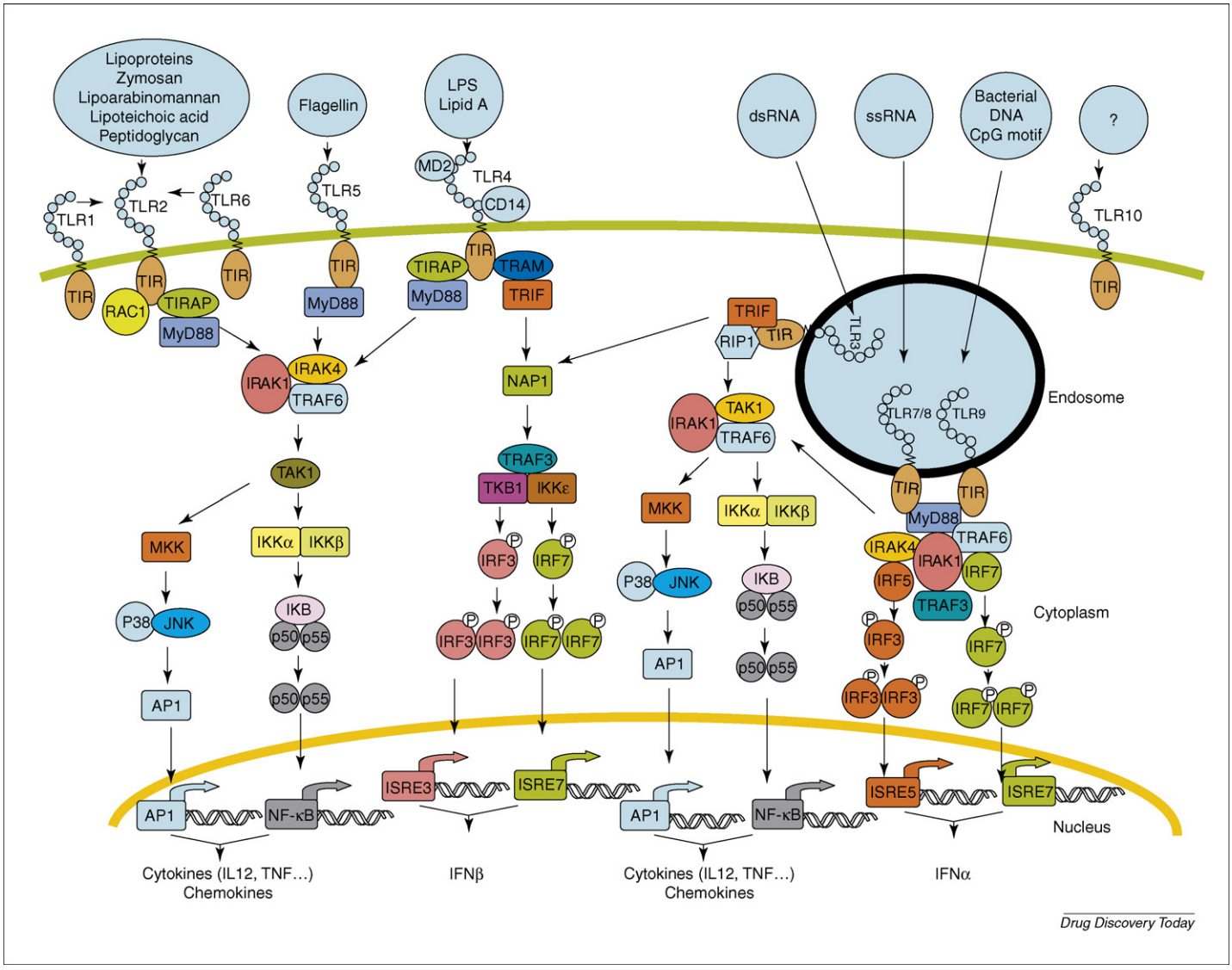

FIGURE 2

Natural ligands and signal transduction of Toll-like receptors. Toll-like receptor (TLR) molecules are type I proteins with a conserved intracellular motif (i.e. TIR domain – responsible for initiation of transduction) and extracellular domain containing a leucine-rich repeat (LRR; symbolized by small circles in TLR), thought to be responsible for ligand binding. TLR2, either alone or in association with TLR1 and TLR6, recognizes cell wall components of bacteria, mycobacteria and fungi. TLR4 has specificity for lipopolysaccharide (LPS). TLR5 is specific for bacterial flagellin. TLR3 is specific for double stranded (ds)RNA, whereas TLR7 and TLR8 are specific for single stranded (ss)RNA – both components of different viruses. TLR9 recognizes DNA oligonucleotide, containing an unmethylated CpG motif, the absence of methylation in CG being a signature of bacterial, and some viral, DNA. All TLRs signal through limited numbers of adapter molecules (e.g. MyD88, TRIF, MAL and TRAM), relayed to the inflammatory pathways: NF-κB, JNK/p38 kinase, interferon regulatory factors (IRF3, 5 and 7). Signaling through TLRs induces the activation and maturation of APCs together with the production of inflammatory cytokines (e.g. type I interferon, AP10, mostly from pDCs through TLR7 and TLR9 and mDCs via TLR3 and TNF-α, and IL12, mostly from mDCs and macrophages through TLR1, 2, 5, 6, 8 and possibly 10), both types of cytokines enhancing mainly TH1 type adaptive immune responses. It is not fully clear how small differences in signal transduction and/or receptor–ligand interaction in different types of cells subtly influence the outcome in terms of the type of cytokine produced. There is some debate on the expression profile of TLRs on cell types other than DCs, such as mastocytes and basophils, and, depending on the agonist used and the cell type targeted in the first place, the cytokine profile and final outcome might be dramatically changed [9,11,48].

Abbreviations: IFN-α, interferon-α; IFN-β, interferon-β; MyD88, myeloid differentiation factor 88; TIR, Toll-interleukin-1 receptor homologous domain; MAL/TIRAP, MyD88-adaptor-like/TIR-associated protein; TRIF, Toll receptor-associated activator of interferon; TRAM, Toll receptor-associated molecule; IRAK, interleukin-1 receptor (IL1-R)-associated kinase; TRAF, tumor necrosis factor-α (TNF-α) receptor-associated factor; TAK, transforming growth factor-β (TGF-β)-activated kinase; NF-κB, nuclear factor-κB; IRF, interferon regulatory factor; ISRE, interferon-stimulated response element, JNK, jun N-terminal kinase; MKK, mitogen-activated protein (MAP) kinase kinase; IKK, inhibitor of NF-κB (IκB) kinase.

variants of this molecule family (i.e. CpG class A, class B and class C) are now being tested and have various effects *in vitro* and *in vivo* [17]. Depending on the nucleotide sequence and length, enabling either formation of duplexes or oligomerization, the different classes of CpG lead to the activation of different cell types and production of different cytokines.

Used in combination with the commercial vaccines Engerix-B® (i.e. for use against HBV) and Fluarix® (i.e. for use against influenza), CPG 7909 (i.e. a class B CpG) showed enhanced immunogenicity without significant toxicity in double-blind Phase I and II trials. CpG 7909 might thereby enable the number or doses of vaccine shots to be decreased [18,19]. The same strategy has been

TABLE 2

Innate immunity receptor agonists in clinical trials

Toll-like receptors (TLRs) as targets for drug development

Company	Drug candidate	Target	Indication	Status
3M	Aldara (imiquimod) cream	TLR7	Genital warts	Marketed
	Aldara	TLR7	Basal cell carcinoma	Marketed
	Aldara	TLR7	Actinic keratosis	Marketed
	Follow-up compound (resiquimod)	TLR7 and 8	Genital warts	Phase II
Anadys	ANA245 (isatoribine)	TLR7	HCV, HBV	Phase II
	ANA975 (oral prodrug of ANA245)		HCV, HBV	Phase II with Novartis
Coley	CPG-7909 (class B CpG)	TLR9	Non-small-cell lung cancer	Entering Phase III with Pfizer
	CPG-7909	TLR9	Cutaneous T-cell lymphoma	Phase II
	CPG-7909 (combination with Rituxan®)	TLR9	NHL	Phase I and II
	CPG-7909, vaccine adjuvant (Engerix-B®)	TLR9	HBV	Phase II
	CPG-10101 (class C CpG)	TLR9	HCV (combination IFN)	Phase I
Dynavax	ISS-1018 (class B CpG) vaccine adjuvant, Engerix-B®		HBV	Phase II
	ISS-1018 (covalently linked with allergen)		Ragweed allergy	Phase II
Idera (ex Hybridon)	Imoxine (hyb 2055) class C CpG	TLR9	Renal-cell carcinoma	Phase II
	HYB 2055 (vaccine adjuvant)	TLR9	HIV	Preclinical
Corixa/GSK	MPL (lipid-A derivative) vaccine adjuvant	TLR4	HBV subpopulation	Marketed
	MPL vaccine adjuvant	TLR4	Papilloma virus	Phase III
	MPL vaccine adjuvant	TLR4	Genital herpes	Phase III
	MPL vaccine adjuvant		Multiple therapeutic vaccine trial (cancer)	Phase I and II
	CRX-675	TLR4	Allergy	Phase I
Ipsen Beaufour	Poly AU (synthetic dsRNA)	TLR3	Breast cancer	Phase II (program discontinued in the 1980s)
Vaxinnate	Fusion protein flagellin–antigen (vaccine)	TLR5	Influenza, HIV	Preclinical

tested with the compound 1018 ISS in a controlled Phase II study [20] with Engerix-B®. This Phase II study demonstrated superior B-cell responses, again suggesting the possibility for reducing injection frequency. A Phase III study is currently ongoing.

Preclinical studies are currently ongoing with most of the available TLR agonists, confirming that most TLR agonists are potent adjuvants for TH1 responses. A recent publication based on the use of the TLR5 agonist flagellin demonstrates that the adjuvant effect is better-achieved if the TLR ligand and the antigen locate in the same endosomal compartments [21]. If this holds true, current stategies, now in preclinical studies, aimed at covalently coupling CpGs, flagellin or imiquimod, a TLR7 agonist, to antigen should be even more powerful. However, because such a strategy requires synthesis of conjugates for each vaccine, it is less practical than just combining two components from a pharmaceutical point of view.

Infectious diseases

Macrophages and DCs, in particular pDCs, are important sources of type I IFN, inflammatory cytokines and chemoattracting chemokines that are triggered by most TLR agonists. Type I IFN has a profound antiviral effect by inducing activation of innate immunity (i.e. γδ T cells and NK cells) and adaptive immunity. To date, mostly TLR7 agonists have been used to target infectious diseases.

TLR7 and TLR8 are the only receptors where new chemical entities (NCEs) have been defined as agonists. TLR7 agonists, for example imiquimod [22] and isatoribine (7-thia-oxoguanosine) [23], are small compounds with structures reminiscent of nucleic acid bases that are present in the natural ligand of TLR7. Agonist molecules of TLR7 and TLR8 have also been synthetized (e.g. the compound resiquimod). Other series of TLR7 and TLR8 agonists that have different pharmacokinetics and distribution profiles have also been produced and might be tested in clinical trials [22,24].

Several hepatitis C virus (HCV) clinical trials have been undertaken with TLR7 agonists. TLR7 agonists essentially target pDCs in humans, and are potent type I IFN inducers. Although there is some debate about the impact of chronic HCV infection on DC *in vivo* because some publications did not find significant alteration of DC function of infected individuals [25], the virus is known to downmodulate type I IFN production from DCs [26], and IFNα, the approved treatment for HCV, reduces viral load. IFNα inducers such as TLR agonists could, therefore, be interesting for use against HCV, particularly if their toxicity profile is favorable compared with that of IFNα. An injectable small-molecule TLR7 agonist, isatoribine, has demonstrated viral-load reductions in IFN-resistant patients in Phase I and II studies [27]. Interestingly, an oral prodrug of isatoribine, ANA975, is in development with complete bioavailability, according to interim Phase I results. This drug,

originally from Anadys, is now co-developed with Novartis. ODN 10101, a class C CpG, is being tested against HBV in a Phase Ib trial.

Imiquimod, used as a cream for topical application, is approved for the treatment of papilloma-virus-induced external genital warts [28]. Of note, imiquimod was used in clinical trials before it was known to be a TLR7 agonist, and the retrospective demonstration of its specificity [29] has largely contributed to the increased interest in the TLR field. Resiquimod, another member of the quinoline family of compounds, targeting both TLR7 and TLR8, has been tested in a double-blind Phase II study against genital herpes, showing a decrease in recurrence rate [30].

Cancer

Several TLR agonists have been used in cancer indications. Although the mechanism of action is not completely understood, it is thought that TLR agonists promote maturation of DCs that can present cancer-specific antigens and production of type I IFN and inflammatory cytokines (e.g. IL12), which in turn promote direct effector function of NK cells, γδ T cells and NKT cells. Both mechanisms might participate in breaking the tolerance towards cancer cells that often secrete immunosuppressing cytokines, and eventually lead, in certain cases, to activation of adaptive immunity.

Imiquimod has been approved for use against superficial basal cell carcinoma [31], and is currently tested for several other cutaneous precancerous and cancerous skin lesions. Double-blind, randomized clinical trials have demonstrated good activity in localized precancerous actinic keratosis lesions [32]. Promising results have also been obtained in localized *in situ* melanoma where surgical resection is contra-indicated [33]. A Phase I clinical trial in metastatic melanoma has also been undertaken, with impressive tumor regression in some patients [34]. It remains to be seen if the potent effect observed with local application of imiquimod can lead to a systemic effect in this indication. It should be noted that, in preclinical mouse models, most of the imiquimod effect is abrogated by removing NK cells, suggesting a prominent role for this lymphocyte subset, indirectly activated by imiquimod through induction of type I IFN and IL12.

CpGs (i.e. TLR9 agonists) are also extensively tested in cancer indications (e.g. renal-cell carcinoma, non-small-cell lung carcinoma, glioblastoma [35] and melanoma) as stand-alone therapies or in combination with various agents. The most significant results were obtained using CPG 7909 in combination with chemotherapy, as reported at the American Society of Clinical Oncology (ASCO) congress. A 112-patient randomized trial showed improved response rates (i.e. 37% versus 19% for chemotherapy alone with $p = 0.048$; and 22% versus 11% for chemotherapy alone after independent adjudication) and a trend toward benefit in median survival (i.e. 12.7 months versus 6.8 months for chemotherapy alone with $p = 0.16$; and 1-year survival of 50% versus 36% for chemotherapy alone). Based on these results, a partnership between Coley Pharmaceuticals and Pfizer has been signed for the development of CPG 7909 in cancer, and a Phase III study has been initiated in combination therapy. Two clinical trials have also been initiated in non-Hodgkin's lymphoma (NHL) with rituximab [i.e. an anti-CD20 monoclonal antibody (mAb)] where the rationale for the combination seems even higher [36]. As a TLR agonist, inducing type I IFN and IL12 can indirectly increase antibody-dependant cellular cytotoxicity (ADCC) by macrophages and NK cells.

For historical reasons, mainly class B CpGs have been tested until now, although with the induction of higher levels of type I IFN and IL12, class C CpGs might have a higher rationale in cancer indications. Clinical trials with class C CpGs are ongoing (e.g. ODN 10101, immunomers [37,38]). It should also be noted that TLRs are expressed in some other cancer types [39]. Therefore, some TLR agonists might act directly on cancer cells, and some signal transduction pathways downstream of TLRs (e.g. TLR3 or TLR4 via TRIF) can lead to apoptosis [40].

Allergy

The final group of indications where TLR agonists have been tested with encouraging results is in the allergy field. In preclinical models, most TLR agonists tested to date produce TH1 inflammatory cytokines, inhibiting production of IL4, IL5 and IL10. Although this could depend on the initial immunological context and/or initial target cells encountered by those agonists (e.g. mastocyte and basophils are TLR-positive and could lead to another type of response), the schemes of administration tested so far in preclinical models have enabled the reversion of TH2 to TH1 responses [41]. 1018 ISS CpG, covalently coupled to ragweed allergen, has demonstrated an improvement in ragweed allergy in controlled Phase II–III trials. Patients treated with this agent experienced a statistically significant reduction in total nasal symptom scores (TNSS), the primary efficacy endpoint of the study, compared with placebo-treated patients in the second year of the trial. Results also showed significant clinical benefit relative to secondary endpoints, including composite hay-fever symptoms and ocular effects, and a significant reduction in antihistamine use. The compound is now entering a large Phase III pivotal trial.

Future challenges in the use of Toll-like receptor agonists

Although the clinical results mentioned here are encouraging in several indications, questions remain to be answered so that a clear rationale about how to use TLR agonists can be formed.

First, although most molecules in development are agonists, several preclinical data show the involvement of innate immunity receptors, such as TLR9 [42] and NKG2D [43], in some chronic inflammatory diseases [10]. Indeed, some self molecular structures are very similar or identical to TLR ligands and, if improperly routed to TLR-containing organelles, can lead to chronic inflammation. This might be an opportunity to treat inflammatory disorders by TLR antagonists in a more specific manner compared with existing agents such as anti-TNF-α, which dramatically decreases those pathologies but results in considerable side effects. TLR4 antagonists (e.g. TAK-242, E-5564) are currently in early-phase clinical development for the treatment of sepsis [44]. The involvement of TLRs in inflammatory disorders raises questions about the tolerance to the agonist products. Usually, the compounds are well tolerated with only mild or manageable inflammation signs, at least when injected or applied locally. However, the number of patients, although beginning to be significant for some compounds, is still low and constant surveillance is still required. This is particularly important considering the tissue distribution of PRRs is not conserved between species, and extra-

polating toxicity data from animal studies is difficult. Anadys recently suspended a Phase II trial with the oral drug ANA975 in HCV because of toxicity seen in animal experiments run in parallel to the clinical trial.

Second, the structure–function relationship between the different agonists and their receptors is not well known. Most of the compounds have been known for years for their immunostimulatory properties and have been used empirically in preclinical models, as well as more recently in clinical trials, before their targets were discovered. Data from knockout mice have established that most TLR ligands are not functional, or are much less functional, in mice lacking the corresponding TLR, but no direct binding of either natural or synthetic compounds to TLRs has been described so far. The first crystal structure of a TLR (i.e. TLR3) has recently been published, revealing a highly glycosylated horseshoe-shaped barrel formed by the LRR, and suggesting that ligand binding could promote dimerization of the receptor, ultimately leading to aggregation of adapters and signaling [45,46]. However, no receptor–ligand interaction could be described in this study. The difference in the activities of class B CpGs (e.g. targeting more B cells, and less pDCs) and class C CpGs (e.g. targeting both B cells and pDCs, thus inducing more type I IFN) might therefore come from differences in different cell type uptake (e.g. because TLR9 is an intracellular receptor), different affinity or oligomerization properties, or pharmacokinetic properties. A recent publication strongly suggests that class A and class C CpGs are routed in the lysosomal compartments because of high-order secondary structures, resulting in IFNα production, whereas class B CpGs with no secondary structures are directed to endosomal compartments leading predominantly to DC maturation and poor IFNα production [47]. The same questions apply for MPL and LPS, both ligands of TLR4 but with different toxicity profiles. These questions are also relevant for other complex ligands such as dsRNA where the pharmacokinetics and degradation are very complex. Furthermore, their large structure might also target other receptors such as retinoic-acid-inducible gene (Rig) or melanoma differentiation-associated gene 5 (MDA-5) [48,49].

Finally, some TLR agonists such as imiquimod have been shown to target not only TLRs but also other important receptors such as adenosine receptors and opioid receptors, and might lead to direct (i.e. nonimmunologically mediated) tumor apoptosis [50,51]. Although preclinical models clearly point towards a strong contribution of immune response to the efficacy of imiquimod, the role of alternative mechanisms of action in humans requires further study.

Thus, to have a sound scientific rationale for the safe and efficacious use of these agonists, a better understanding of the downstream molecular pathways and the exact profile of biological activities of the different agonists, together with precise knowledge of the tissue distribution, metabolization and cell uptake, is still awaited.

Concluding remarks

From their mechanism of action, innate immunity receptor agonists have an obvious potential interest as vaccine-adjuvant or anti-infectious agents. TLR agonists have already demonstrated their value in these indications as vaccine adjuvants (e.g. MPL – a vaccine adjuvant for HBV) and stand-alone therapies (e.g. imiquimod – a cream for the treatment of genital warts). Encouraging results in early-phase clinical trials in HBV and HCV show that TLR agonists could be important in other therapeutic fields such as cancer and allergy, with promising results in some late-stage clinical development trials (e.g. controlled Phase IIb in pulmonary cancer and allergy for TLR9 agonists), and also a marketed drug, imiquimod, in basal-cell carcinoma, a drug with potential for other cutaneous cancer. Many early-phase clinical trials are now ongoing with different TLR agonists in various indications and the results obtained so far with crude compounds (i.e. without a lot of medicinal chemistry input except for TLR7 and TLR8 agonists) are encouraging. With the rapid increase of knowledge regarding the molecular structures of the receptors, their transducing elements and the complex interaction between cells bearing these receptors more rational use of agents with improved pharmacological properties, alone or in combination with other treatments, promises a successful future.

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